newfood

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Issue 1 · 2014

Reducing the total and saturated fat of baked goods

Charles Speirs, Bakery Science Manager, Campden BRI

Risk assessment and validation in frozen food manufacturing

Lilia M. Santiago-Connolly & Raghu Ramaswamy, Heinz North America

Why ISO 21469 is on the rise

Ashlee Breitner, Business Unit Manager, NSF International

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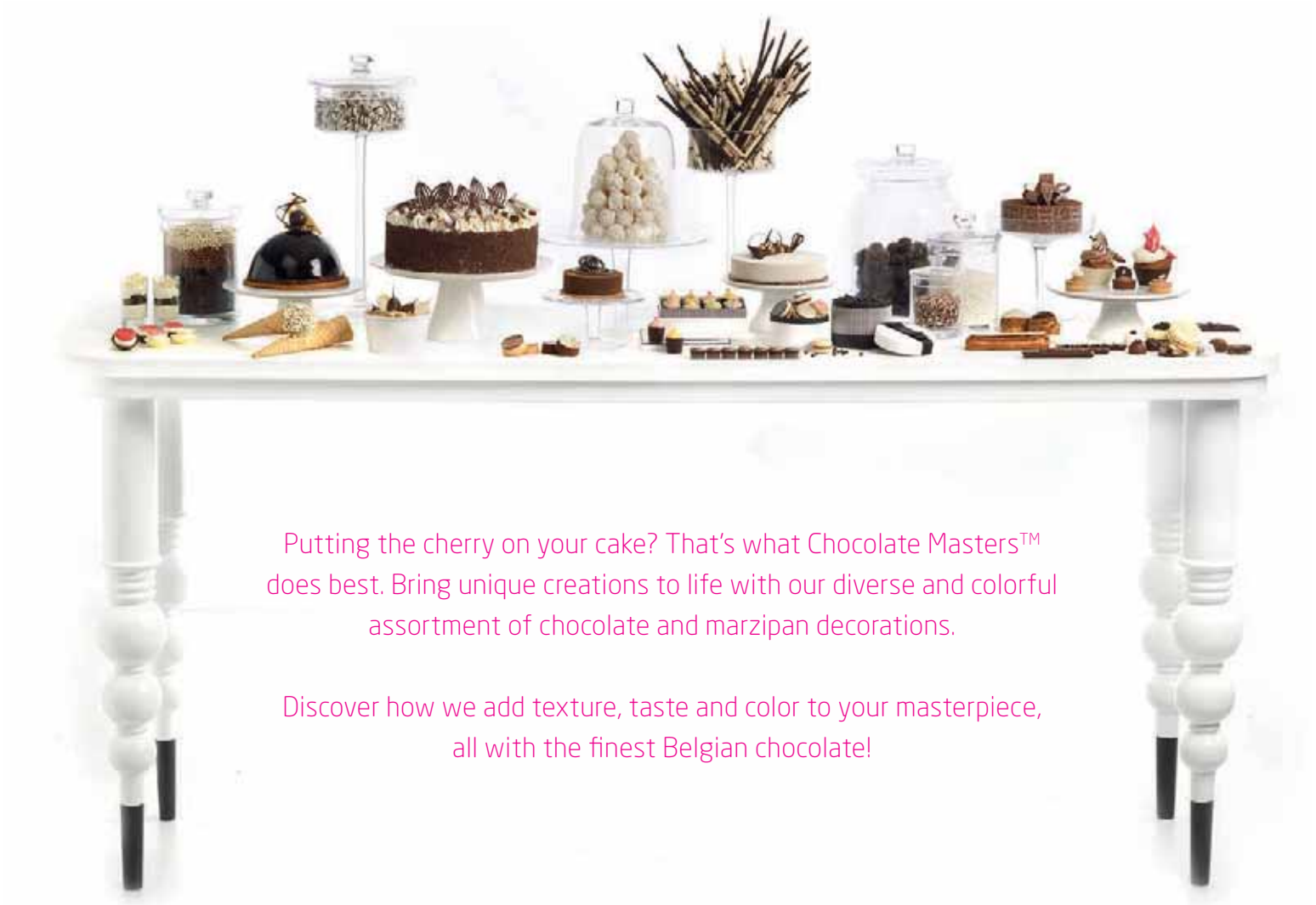

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SURPRISING DECORATIONS: small extras for a BIG impact

Extra appealing touch

Barry Callebaut introduces new and innovative decoration products in answer to the latest market trends in food, by expanding its product range with Cake Collars, Brazilian Pencils, Shiny Cocoa Nibs and Crispy Mignature™ range, enabling its customers to give their end products an extra finishing touch in the most convenient and easiest way.

Cake Collars – more personalization with the new marzipan bands

With its recently launched Cake Collars, Barry Callebaut is responding to the personalisation trend: Today's consumers are looking for products that are unique in texture, colour and flavour. Cake Collars are flexible and adaptable marzipan bands. These smart, cost-effective and easy-to-use, decorative ribbons have both a new fresh looking design and can be personalized or tailor made.

Brazilian Pencils – bringing the 2014 FIFA World Cup™ Brazil to the chocolate world

The market desire for unique, customised products while also taking into consideration customer's limited time and budget resources are also being answered with the launch of the limited edition Brazilian Pencils. These smart chocolate decorations in the colours of the Brazilian flag are introduced on the occasion of Brazil hosting the 2014 FIFA World Cup™ Brazil. The can be used to conveniently decorate bakery, ice cream and desserts. In order to decorate smaller pastries such as cupcakes, the pencils can be broken into smaller pieces.

Shiny cocoa nibs – adding a special indulgence moment

Barry Callebaut also unmasks 'Virtuous Simplicity' as a clear consumer trend. This means that consumers, struck with a multitude of food choices, in the end like to choose natural, simple and healthy products. The shiny cocoa nibs, crunchy pieces of pure roasted and cocoa kernels that are broken into smaller pieces, are a perfect example of such a product. Moreover, the shiny cocoa nibs have a refined and intense roasted taste and offer a pleasant, mild crunch. Their deep chocolate colour, and appealing shiny chocolate gloss are most attractive. Cocoa nibs add a delicate taste and hand-made look to confectionery, desserts, pastries, ice cream coatings, breakfast cereals, cereal bars and can be baked into biscuits, rolls and breads.

Crispy Mignature™ – the perfect tool for "My Daily Luxury"

The new Crispy Mignature™ range are caramelised mini pieces of hazelnut, almond or nibs with a natural, roasted flavour and pleasant caramelly sweetness. In the spirit of the so called 'My Daily Luxury' consumer trend, Barry Callebaut succeeds to offer its customers a unique

multi-sensory experience tool they are looking for. Indeed, there is an increasing demand for top-notch quality, but also for pure enjoyment at the same time. The Crispy Mignature™ range offers a 100% natural crunch in an appealing mini-size. They are ideal as one shot -inclusions in confectionery and bakery applications as to add crunchiness in a filling or a dough in one go. On the other hand, they can also serve as a decoration, sprinkled onto soft glazing, jelly or on chocolate.

"By continuously looking for innovative convenient product solutions to serve

our customers' needs, Barry Callebaut is keeping up to date with the latest consumer market trends in food. The novelty chocolate decorations of Barry Callebaut are a great example of how market research is translated into convenient and smart customer products," Sofie De Lathouwer, Marketing Director FM Western Europe at Barry Callebaut concludes.

For more information: www.barry-callebaut.com

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Schedule

New Food is published bi-monthly (six times per annum) and is available by subscription at £90.00 for a year which includes on-line membership access. Back issue copies can be requested at £15.00 per copy.

Subscriptions

Subscription enquiries to khutchinson@russellpublishing.com or telephone +44 (0) 1959 563311

Publishers

New Food is published by:
Russell Publishing Ltd, Court Lodge,
Hogtrough Hill, Brasted,
Kent, TN16 1NU, UK
Telephone: +44 (0) 1959 563311
Fax: +44 (0) 1959 563123
Email: food@russellpublishing.com
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Circulation

New Food can guarantee its circulation is 13,594 (for the six issues distributed between 1 July 2012 to 30 June 2013). The publication is ABC audited. This is an independent verification that our circulation is genuine.

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Printing by wyndeham grange

Published March 2014

Helen Bahia
Editor

Important issues in 2014

As we look towards important issues for the food and beverage industry in 2014, we start with our regular supplements on food safety and food grade lubricants. Francois Bourdichon, *New Food* Editorial Board Member and Corporate Food Safety, Microbiology and Hygiene Manager at Barry Callebaut, looks at *Listeria monocytogenes*, questioning what we've learned about the pathogen and its effects over the past 30 years. While *Listeria* is responsible for fewer infections than *Salmonella* or *Campylobacter*, its high mortality rates and higher hospitalisation rates ensure that food and beverage manufacturers have to be on alert to ensure that all foods are analysed to ensure complete safety from the disease. In our other article, Lilia Santiago-Connolly and Raghu Ramaswamy from Heinz North America look at the need for risk assessment and validation in frozen food manufacturing. While freezing slows down the growth of microorganisms in food, it does not necessarily inactivate them. The authors discuss the need for food manufacturers to ensure that foods are safe from microorganisms that can prove dangerous to consumers prior to freezing. Please turn to page 17 for our in-depth look at food safety.

Ashlee Breitner, Business Unit Manager at NSF looks at ISO 21469 and how the recent uptake of global food safety standards has prompted the food safety supply chain to implement more rigorous product safety measures and how ISO 21469: 2006 (E) affects food grade lubricant manufacturers and users. Please turn to page 39 for our in-depth look at food grade lubricants.

As always, if you have any comments or would like to contribute an end-user article or submit news, please contact me directly at hbahia@russellpublishing.com. In addition, don't forget to join our groups on LinkedIn and Twitter, details are opposite.

New Food is proud to be affiliated with EFFoST, The European Federation of Food Science & Technology, a non-profit association that federates food science and technology organisations in Europe.
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Contents

Events

MARCH

World Food Technology & Innovation Forum
Date: 3 – 4 March 2014
Location: St. Paul's, London, UK
e: enquire@wtgevents.com
w: www.foodinnovate.com

Contamination Control Seminar
Date: 4 March 2014
Location: Chipping Campden, Gloucestershire, UK
e: information@campdenbri.co.uk
w: www.campdenbri.co.uk/contamination-control-seminar.php

4th Annual Food Manufacturing & Safety Forum
Date: 10 – 11 March 2014
Location: Dallas, Texas, USA
e: enquire@wtgevents.com
w: www.foodmanufacturingsummit.com

Foodex 2014
Date: 24 – 26 March 2014
Location: NEC Birmingham, UK
e: emma.pellman@wrbm.com
w: http://www.foodex.co.uk

Food Structure and Functionality Forum Symposium from Molecules to Functionality
Date: 30 March – 2 April 2014
Location: NH Grand Krasnapolsky, Amsterdam, The Netherlands
w: www.foodstructuresymposium.com

RME2014 – 9th conference on food, feed & water analysis
Date: 31 March – 2 April 2014
Location: Noordwijkerhout, The Netherlands
e: RME@bastiaanse-communication.com
w: www.bastiaanse-communication.com/RME2014

APRIL

Food Technology and Innovation Forum 2014
Date: 7 – 9 April 2014
Location: Chicago, Il, USA
e: enquire@wtgevents.com
w: www.thefoodsummit.com

15th Food Colloids Conference
Date: 13 – 16 April 2014
Location: AkademieHotel Am Rüppurrer Schloß 40 76199 Karlsruhe, Germany
e: info@ift.org
w: www.ift.org

MAY

Seafood Expo Global
Date: 6 – 8 May 2014
Location: Brussels, Belgium
e: customerservice@divcom.com
w: www.seafoodexpo.com

Vitafoods Europe 2014
Date: 6 – 8 May 2014
Location: Geneva, Switzerland
e: amy.ford@informa.com
w: www.vitafoods.eu.com/lsnewfood

IAFP's European Symposium on Food Safety 2014
Date: 7 – 9 May 2014
Location: Budapest, Hungary
e: info@foodprotection.org
w: www.foodprotection.org/europeansymposium

XII International Conference on the Applications of Magnetic Resonance in Food Science: Defining Food by Magnetic Resonance
Date: 20 – 23 May 2014
Location: Cesena, Italy
e: foodmr2014@unibo.it
w: www.foodmr.org

3rd International ISEKI-Food Conference
Date: 21 – 23 May 2014
Location: Athens, Greece
e: office@iseki-food.net
w: www.isekiconferences.com/athens2014

JUNE

15th Joint Fera/JIFSAN Annual Symposium: Emerging Issues in Food Safety
Date: 9 – 11 June 2014
Location: The Food And Environment Research Agency, Sand Hutton, York, UK
e: fera_jifsan2014@fera.gsi.gov.uk
w: www.fera.co.uk/events/jifsan2014

Salt-Sugar-Lipids Reduction
Date: 17 – 18 June 2014
Location: Nantes, France
w: www.pleasure-fp7.com/conference/index.html

IFT Annual Meeting 2014
Date: 21 – 24 June 2014
Location: New Orleans, Lousianna, USA
e: info@ift.org
w: www.am-fe.ift.org/cms

10th EPRW – European Pesticide Residue Workshop
Date: 30 June – 3 July 2014
Location: Convention Centre, Dublin, Ireland
e: info@eprw2014.com
w: www.eprw2014.com

AUGUST

IAFP 2014
Date: 3 – 6 August 2014
Location: Indianapolis, Indianna, USA
e: info@foodprotection.org
w: www.foodprotection.org/annualmeeting

IUFoST 17th World Congress
Date: 17 – 21 August 2014
Location: Montreal, Canada
e: carole@iseventsolutions.com
w: http://iufost2014.org

7th International Congress on Biocatalysis – biocat2014
Date: 31 August – 4 September 2014
Location: Hamburg, Germany
w: http://biocatconference.de

SEPTEMBER

Food Micro 2014
Date: 1 – 4 September 2014
Location: Nantes, France
w: www.foodmicro2014.org

7th International Whey Conference
Date: 7 – 9 September 2014
Location: Rotterdam, the Netherlands
w: http://www.iwc2014.com

If you have a diary event you wish to publicise, send details to Martine Shirtcliff at: mshirtcliff@russellpublishing.com

Sanitary design NIR for food analysis

At-line NIR – DA 7250 SD

The DA 7250 SD NIR is designed for use in food production. Its stainless steel IP65 certified casing allows it to be placed anywhere, and the open analysis area and smooth corners make it very easy to clean. Advanced diode array NIR technology provides accurate analysis of moisture, protein, fat and more in only six seconds. All types of food products are analysed with little or no sample preparation – meats, butter, cream, powders and baked products are a few examples. Optional disposable cups completely remove the need for cleaning between samples.

USDA Approved In-line NIR – DA 7300 SD

The DA 7300 continuously measures moisture, fat, protein and more in your process, in for example pipes or chutes. It is officially approved by the USDA for measurement of meat and dairy products, and has many additional food applications.

The analysis results are sent to a plant's process control system for automatic plant optimisation or are read out to a display screen for manual adjustment. The DA 7300 incorporates a real-time video camera with separate illumination for a unique view directly into the process. The video can be viewed on a computer attached to the system's network – be it in the same building or thousands of miles away.

www.perten.com

PureLine PQ UV system from Hanovia specifically designed for food and beverage applications

Designed specifically to provide validated, performance qualified (PQ) and chemical-free UV treatment of water in the food and beverage industries, Hanovia's PureLine PQ UV system takes disinfection to another level.

The PureLine PQ has not only been designed to exacting industry standards using the latest design techniques to maximise performance, but its disinfection performance has also been independently tested and validated by Carollo Engineers in the USA to the 2006 USEPA protocol following the UV Disinfection Guidance Manual (UVDGM) guidelines. It is approved to most international standards and material certificates are available for all the FDA-compliant wetted parts.

A revolutionary feature of the PureLine PQ is its optimised, absolute intensity UV monitor. Located within the chamber, it allows the controller to automatically correct the dose calculation as UV transmittance varies without the need for an external UV transmittance monitor. In addition, the factory-calibrated dry UV sensor provides absolute UV intensity monitoring in real-time and, because it remains outside the water flow, it can be removed and inspected without interrupting the process. Its patented shutter port means there is no risk of UV exposure and no need for the operator to wear safety equipment while performing the field verification. Watch our PureLine PQ video on our YouTube Channel.

www.hanovia.com

Peter Ragaert and An Vermeulen
Pack4Food

Mieke Buntinx and Roos Peeters
Research Group Packaging Technology & VerpakkingsCentrum, University Hasselt

New research gives further insights on O_2-ingress in food packaging

Diversity in food packaging has become increasingly important in the last few decades due to different trends such as globalisation and convenience. This has resulted in an increased need for certain barrier properties in order to guarantee the desired shelf-life of the packaged food product. In the case of gas barrier properties, many food products need to be protected from oxygen, on the one hand making this parameter very important in evaluating new materials (e.g. bioplastics) for food packaging applications and on the other hand, there is an increased attention for investigating the influence of different processing techniques on the barrier properties of packaging materials.

A frequently used process in the food packaging industry is thermoforming. During thermoforming, an (inline) extruded sheet is heated to its softening temperature and subsequently deformed through application of mechanical stretching and/or pressure into a tray. This process directly impacts several properties of the sheet. One of these properties, especially in the case of food packaging under modified atmosphere (MAP – Modified Atmosphere Packaging), is the oxygen permeability. As a result of the increased surface area and thinning of the sheet, the OTR (Oxygen Transmission Rate) of the tray will be increased. However, various studies have shown that OTR values of the virgin material cannot easily be extrapolated to thermoformed packaging[1-3]. One of the reasons might be a poor control of the material thickness

distribution in the walls, the corners and/or the bottom, which is a major drawback of thermoforming. On the other hand, the effect of physical thinning on the OTR can sometimes be counteracted by reorientation, closer chain packaging and restriction of chain mobility of amorphous polymer chains during deep drawing or stretching of the material. Reorientation and crystallisation is known to be related to altered sorption and diffusion of oxygen in polymer materials, and might cause a decrease of the OTR[4].

Recently, the research groups Packaging Technology & VerpakkingsCentrum and Applied Analytical Chemistry of the University of Hasselt have conducted the Flanders' FOOD MaProDe_Ox project in collaboration with companies from the Belgian food and packaging industry. The aim was to evaluate and quantify the impact of the thermoforming process on the OTR of several selected commercial packaging materials.

Figure 1: Each packaging material was deep drawn into 3 trays with top dimensions of 190x132 millimetres and variable depths of 25 millimetres, 50 millimetres and 50 m millimetres/r5 (round corners) (top to bottom)

In this project, commercial sheet materials of monolayer polypropylene (PP); PP/ethylene-vinyl alcohol co-polymer/PP (PP/EVOH/PP); polystyrene/EVOH/polyethylene (PS/EVOH/PE); amorphous polyethylene terephtalate/PE (APET/PE); and APET/PE/EVOH/PE were thermoformed into three trays with the same top dimension of 190x132 millimetres and variable depths of 25 and 50 millimetres (radius one millimetre), and a variable radius of five millimetres for the corners of the 50 millimetre tray (Figure 1). The thickness of the sheets ranged between 350 and 400 µm. The EVOH content in the three multilayers was determined to be on average three

Table 1: OTR of thermoformed trays expressed in [cc/package • day • atm] (n=2-3)

OTR [cc/package • day • atm]	Tray 25 mm radius 1 mm	Tray 50 mm radius 5 mm	Tray 50 mm radius 1 mm
PP	6.5 ± 0.4	9.3 ± 0.2	11.0 ± 0.5
APET/PE	0.52 ± 0.06	0.87 ± 0.01	0.97 ± 0.07
PP/EVOH/PP	0.067 ± 0.001	0.161 ± 0.002	0.152 ± 0.007
APET/PE/EVOH/PE	0.088 ± 0.019	not done	0.120 ± 0.005
PS/EVOH/PE	0.053 ± 0.007	not done	0.095 ± 0.010

per cent (the EVOH type used contained 32 mol% ethylene). The thickness distribution after thermoforming into three different trays was measured in detail and visualised showing the regions that were most affected by the deep drawn process for all test materials. **Figure 2** shows an example of the 50 millimetre PP tray.

The oxygen transmission rate (OTR) of the sheets and the trays was measured using a Mocon Ox-Tran 2/21 in accordance with ASTM F-1927 and ASTM F-1307 respectively. The OTR of the trays increased with the drawing depth (**Table 1**, page 9). Increasing the radius of the corners in the 50 millimetres tray showed a beneficial effect on the OTR of PP and APET/PE. This study also confirms the excellent oxygen barrier properties of EVOH. The results show that around three per cent EVOH in the different multilayer materials is sufficient for deep drawing to depths of 25 and 50 millimetres. The OTR of the PP/EVOH/PP, APET/PE/EVOH/PE

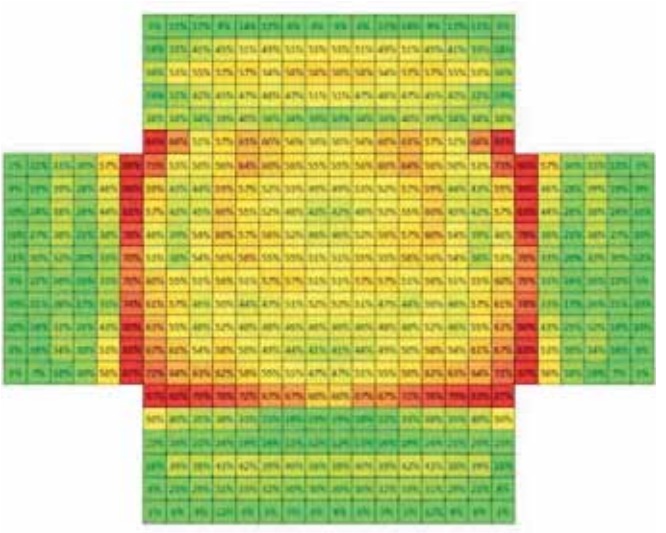

Figure 2: Percentual thinning in bottom, walls and corners as a consequence of plug-assisted deep drawing the PP sheet into a tray with 50 millimetre depth (radius one millimetre)

and PS/EVOH/PE sheets were in the same range (0.9-3.2 cc/m^2.day.atm). The OTR of a 25 millimetre and 50 millimetre PP tray can be improved with a factor of 96 and 73 by the presence of an EVOH layer (around three per cent) respectively. The OTR of the 25 millimetre and 50 millimetre APET/PE/EVOH/PE trays was six and eight times better than the respective trays without an EVOH layer (**Table 1**, page 9).

More detailed results of this study indicate that the calculated OTR based on a homogenous material distribution, can be used as a rough approximation of the real OTR. However, detailed analysis of unequal thinning, orientation and crystallisation remains necessary to explain the deviation of the measured OTR as compared to the predicted one. Certainly in the case of monolayer PP, there is potential for improvement of the OTR as compared to the predicted value, due to reorientation of PP polymers during deep drawing.

The significance of the OTR values obtained in this study requires a translation in terms of the oxygen level in the package that will finally determine the shelf-life of the product in a modified atmosphere packaging. The O_2-concentration in the packaging is thereby determined by *(i)* the residual O_2 after packaging which is dependent on the filling-system and *(ii)* the O_2 ingress during the shelf-life (OTR). On technical sheets the OTRs are often mentioned at 20-25°C while a lot of packed food products are stored in the cold chain. Therefore it is difficult to estimate the permeability of the packaging configuration under realistic storage conditions. To fill these gaps the software PredOxyPack® (www.predoxypack.com) has been developed in collaboration with Food2Know based on previous results of a Flanders' FOOD project. The validation of this model was performed for monolayer and multilayer films and for the combination of bottles and caps, taking into account different temperatures. This validation is thoroughly discussed in Van Bree *et al*[5].

PredOxyPack® allows the user to predict the oxygen ingress for different packaging configuration, materials and time-temperature profiles. In this way, realistic estimations can be made for the circumstances to which the food is exposed during its preservation including cold chain conditions. For the development of PredOxyPack®, a lot of attention is paid to the user-friendliness by among others *(i)* the incorporation of predefined packaging configuration for which the exchange surfaces and volumes are automatically calculated, *(ii)* the automatic unit conversions and *(iii)* a build-in permeability database of different packaging materials with minimum, maximum and default values of OTRs. Besides, users can also choose a custom packaging (e.g. pouch).

Once the user has chosen the packaging configuration, the composition of the packaging material can be inserted for each packaging component separately. The software easily permits inserting or deleting an extra layer. Next to the polymer type, the user has to indicate the OTR, test thickness and test temperature of the polymer. These data can be either filled in by the user based on technical sheets or automatically appear in the software based on a build-in database with default values for each polymer type. Finally the user has to fill in the actual layer thickness of the polymer in the multilayer. Based on this information, the residual oxygen level after filling and the time temperature profile to which the packed product is exposed during filling, transport and storage, the oxygen evolution in the package can be calculated.

The output screen of the software (**Figure 3** and **Figure 4**, page 11) represents a graphical output of the simulations (% or ppm O_2 in the headspace as a function of time) together with the followed temperature

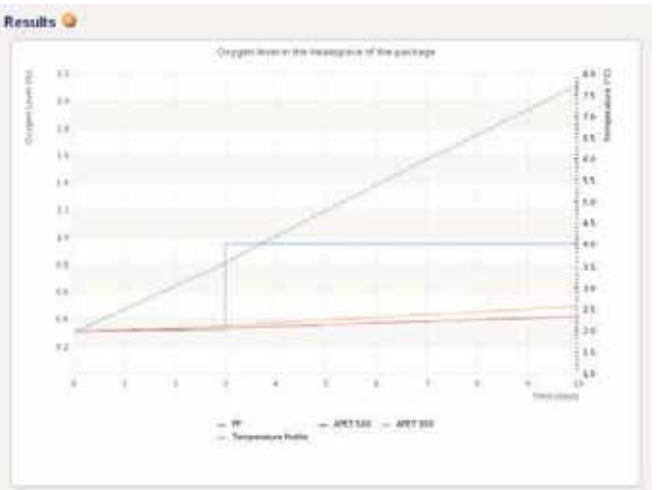

Figure 3: PredOxyPack® output: % O2 as a function of time in the headspace of a PP or PET tray with PE/EVOH/PE top film stored for three days at 2°C followed by seven days at 4°C

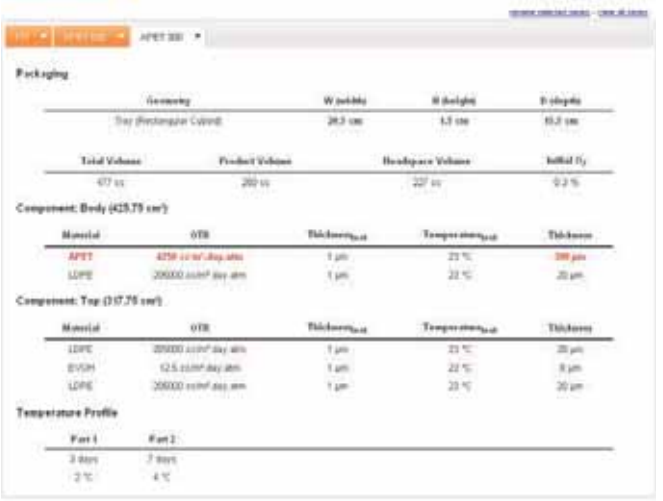

Figure 4: PredOxyPack® output: Summary of all input variables for the performed simulations

profile. Next to this graph, a table with the summary of the input values is mentioned. This is important when a user wants to reopen previous performed simulations.

PredOxyPack® is used by among others food business operators (FBO), converters, packaging designers. It can help FBO to make the translation from the oxygen permeability which is mentioned on a technical sheet towards their specific packaged product during the reasonable foreseen conditions of storage e.g. within the cold chain. PredOxyPack® can give the FBO also a quick outcome on the comparison of different materials (based on the technical sheets) provided by one or different suppliers for the realistic conditions of the own packed product. With the software tool, the FBO can quickly compare new, innovative packaging materials and make sure that the required oxygen barrier can be achieved. Because of the various options regarding material choices, packaging design, time and temperature combinations, it is possible to explore in a first phase of the development a wide range of packaging options through the software tool without additional costs of testing all the materials. Based on these outcomes, the most promising concept could be analysed in a second phase with oxygen permeability measurements.

Regarding new upcoming materials, the build-in permeability database of PredOxyPack® will be updated regularly. Recently, an IWT-funded research project at Ghent University in collaboration with different research institutes (University College Ghent, Verpakkingscentrum, Belgian Packaging Institute and the technology centre of Flanders' Plastic Vision) and 22 companies has been performed on the applicability of bioplastics for food packaging, including modified atmosphere packaging (MAP). Different packaging materials from renewable biobased resources were investigated for different properties such as O_2- and H_2O-permeability (**Table 2**), mechanical properties and seal properties[6]. **Table 2** shows a high diversity in properties between the investigated materials, indicating that different types of food products can be packed, including those products that require a good O_2- and/or H_2O barrier. Furthermore, the obtained data in **Table 2** are in the same range of different conventional food packaging materials such as OPP, PET/PE, OPA/PE, from a technical point of view making the investigated bioplastics valuable alternatives to those conventional materials.

The different materials have been subsequently tested in elaborated storage experiments with short (e.g. fresh red meat), middle-long (e.g. sliced meat products, grated cheese) and long shelf life food product (e.g. rice cakes). Tests with MAP-packaging of short and middle-long shelf-life food products showed amongst others that the barrier properties of different bioplastics were sufficient in order to maintain the desired gas mixture during the complete shelf-life period inside the packaging. This shows that MAP-applications by using bioplastics are possible. Therefore, these type of materials could also be considered as packaging materials for MAP-applications. It should be mentioned that the future use of bioplastics will depend, next to the technical performance, on the price evolution of these materials as well as transparent waste management options.

The future use of bioplastics will depend, next to the technical performance, on the price evolution of these materials as well as transparent waste management options

Table 2: O_2- and H_2O-permeability of different bioplastics-packaging materials, measured on the actual thickness of the film/tray (from Peelman *et al.*, 2013)

Material (Tradename)	O_2 (cc/m².d) 23°C – 75% RH	H_2O (g/m².d) 38°C – 90% RH	Thickness (µm)
Natureflex™ type 1	9.9	10.1	55
Natureflex™ type 2	3.4	5.0	44
Ecoflex+Ecovio/Ecovio/ Ecoflex+Ecovio	815.0	216.4	55
Metallised PLA	25.4	2.3	20
Cellophane™ /Metal/PLA	9.1	9.7	46
Paper/AlOx/PLA	45.7	6.0	91
Bioska (multilayer PLA)	617.6	275.1	34
Natureflex™ type 1/PLA	11.01	11.3	60
PHB/Ecoflex	142.1	80.6	87
Xylophane A (coated on paper)	3.7	24.3	100
PLA tray (Ingeo)	46.8*	3.8	200-300

* 50% RH inside; 0% RH outside; measured at 23°C

Research described in this publication was supported by grants from:

- Flanders' Food, Belgium. MaProDe_Ox project: Impact of Material, Process and Design on the Oxygen Permeability of Thermoformed Packaging
- IWT (Institute for the Promotion of Innovation by Science and Technology in Flanders, Belgium) and 22 companies. IWT-CO 095062 Application of bioplastics for food packaging
- UGent. IOF10/StarTT/025. Development and commercialisation of a software package to predict the O_2 ingress in packages for food products

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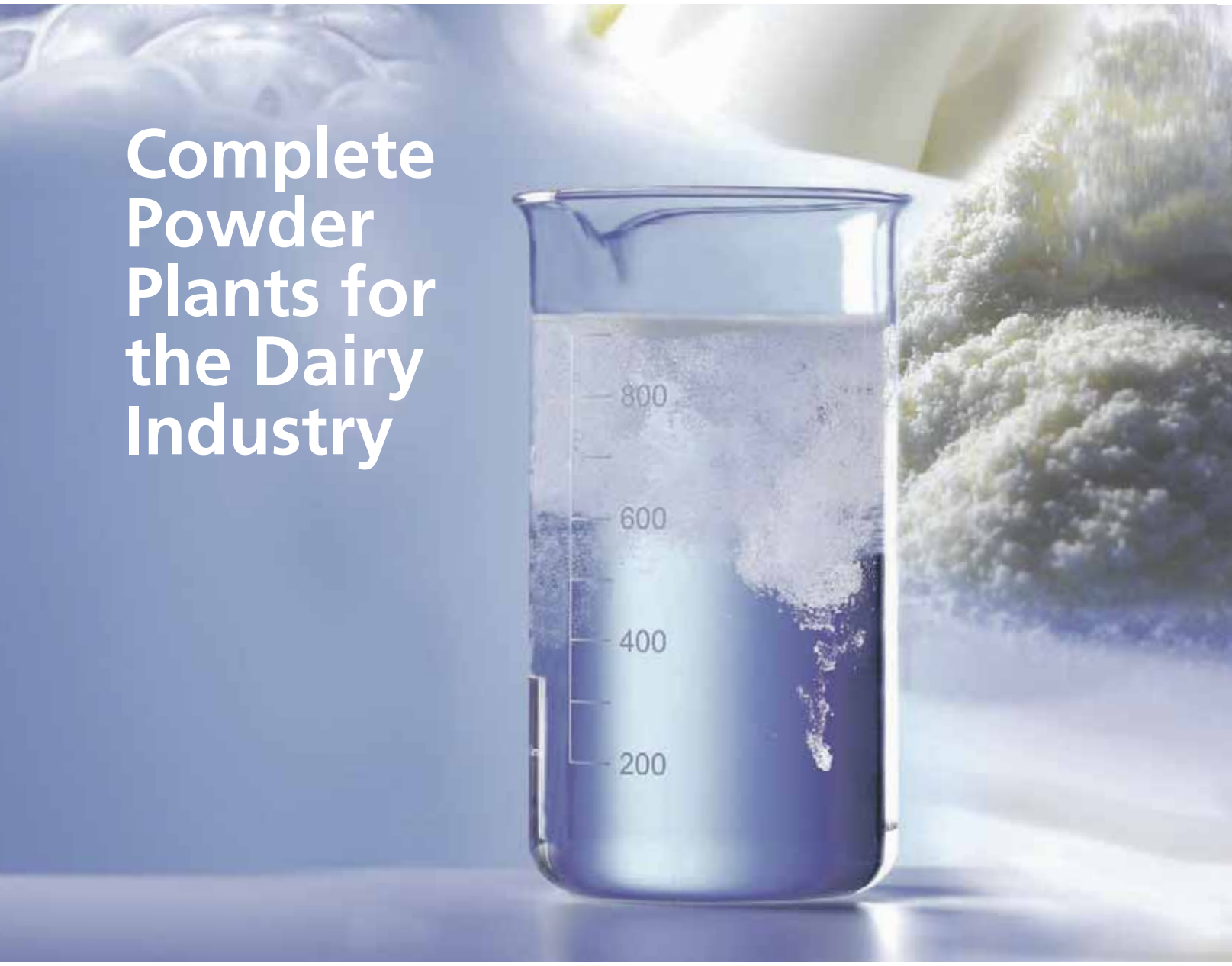

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Advanced microbial modelling techniques and risk-based management applied to aseptic-UHT process

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Ultra High Temperature (UHT)-type products are ambient stable products, with a long shelf life (three to six months). Since they do not require any cold chain storage and can be consumed immediately, they are consumed extensively everywhere on the globe. They are defined as commercially sterile meaning that the product "must be free of microorganisms capable of growing under normal non-refrigerated conditions of storage[1]." Basically, the challenge in a UHT process is to fill a 'sterile' product in a 'sterile' container in order to achieve commercial sterility.

Traditionally, this complex process has been run using deterministic or empirical process settings. To move towards science-based process settings, risk of microbial contamination along the whole aseptic-UHT process must be quantified. That is now feasible due to modelling techniques dedicated to the microbial contamination variations encountered in the process, and also to the advanced probabilistic techniques enabling to take the inherent variability and uncertainty of biological processes into account. Such techniques offer the possibility of improving on the currently tolerable commercial sterility failure rate (less than one defect per 10,000 units produced). In addition, benefits of applying a risk-based management system are: i) to implement process settings in a transparent and scientific manner instead of empirically; ii) to develop a uniform common structure whatever the production line, leading to a harmonisation of these process settings, and; iii) to quantify the impact of various management measures and weigh them against their implementation cost.

The aseptic-UHT process is relatively complex but can be defined in three main phases based on the material and process flow (**Figure 1**, page 14)[2]. The first one is a 'non-sterile' phase, in which product ingredients and packaging raw materials are received and sterilised. A UHT treatment is applied to the product, and thermal and / or chemical sterilisation treatments are applied to the packaging. The second one is a phase where the product and packaging have to be 'sterile' and aseptically filled and sealed. This phase includes the most challenging steps in terms of controlling contamination risk to the product since biofilm formation, air recontamination and defective sealing can occur and cause a significant number of product sterility failures. The last phase encompasses the end product storage up to the consumer place.

Risk analysis is a systematic and comprehensive methodology to estimate the risk associated with a complex process[3] and is split into three pillars: microbial risk assessment (MRA), risk management, and risk communication. For an aseptic-UHT food process with a commercial sterility target, the four steps of MRA can be defined as can be seen in **Figure 1**[2] on page 14. The hazard identification consists of identifying microbiological agents capable of causing adverse health effects (for pathogens) or significant sensory deterioration of the product (for spoilage agents). The hazard characterisation is the evaluation of the nature of the adverse effect of pathogens (i.e. public health concern) and spoilage agents (i.e. spoilt product). The exposure assessment consists of the quantification of contamination along different pathways through the whole process up to consumption. Finally, the risk characterisation is the quantification of the risk in terms of public health (for pathogens) and the probability ('risk') to get a commercial sterility failure (for spoilage agents).

Modelling microbial variations

Mathematical models enable the quantification of microbial variations at each aseptic-UHT key process step – from raw materials reception up to end-product storage as illustrated in **Figure 1** (page 14). The microbial variations include reduction at the sterilisation step (for both product and packaging lines) and increase (due to growth or recontamination). In aseptic-UHT process, all contamination variations need to be considered due to the respect of the commercial sterility of the product, even if the probability of occurrence is very low.

The microbial reduction is due to the UHT treatment of the product, the sterilisation of the packaging or the cleaning procedures. The most commonly applied models in thermal treatment are the log-linear reduction, using the D and z concepts[4]. For packaging sterilisation, there are two processes which can be either coupled or separated5. The first one consists of heat treating the packaging with saturated steam or with a combination of super-heated steam and hot air; alternatively the packaging can be heated by extrusion using a form-fill-seal packaging

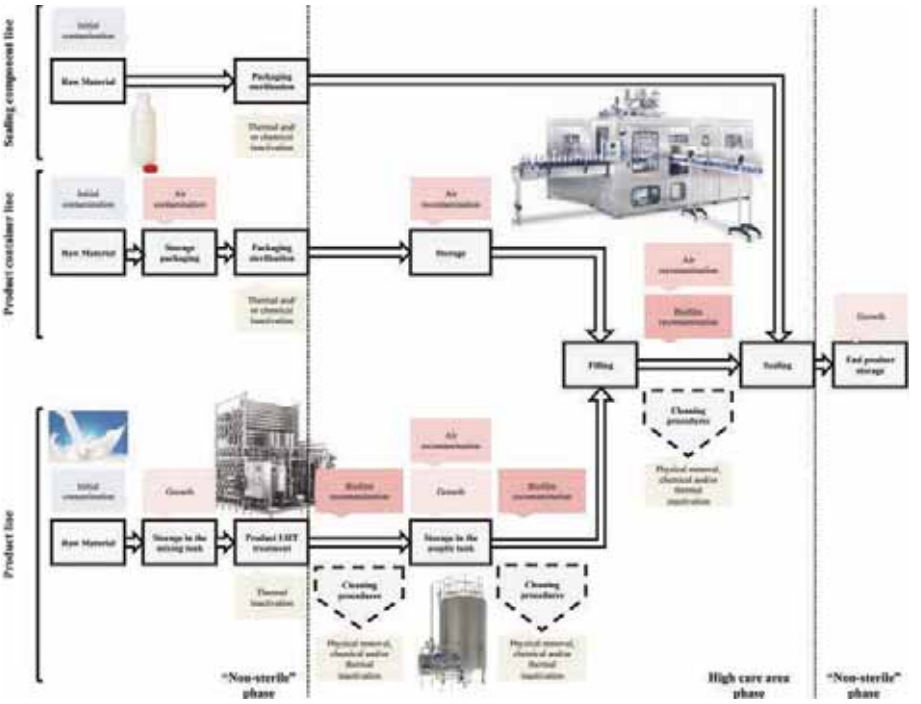

Figure 1: Aseptic-UHT food process from the raw materials to the final end product storage and their possible associated microbial variations. Blue squares correspond to the initial contamination, green squares represent the microbial reduction and the red squares symbolise the possible microbial increase

system. The second process consists of applying a chemical treatment, either hydrogen peroxide or peracetic acid. Generally, the mathematical models developed to predict the probability of surviving packaging sterilisation are based on a log-linear reduction pattern[2,5]. In aseptic-UHT lines, the cleaning procedures are generally split into three sequential phases: i) Cleaning-In-Place (CIP), which is an application of a chemical agent, either acid or alkaline; ii) Hot water rinsing applied to equipment surfaces; or iii) Sterilisation-In-Place (SIP), which is the re-sterilisation of the line[6]. A few mathematical models describe cleaning operation exits[7], but their implementation in MRA models is still at an early stage.

Concerning the microbial growth, in predictive microbiology, primary models describe the log count variation over the time and secondary models the effect of storage temperature and product formulation (e.g. pH) on the lag and maximum growth rate[8]. In the case of growth after the heat-treatment process (e.g. storage in aseptic tank), it is sensible to consider that only one, or a few, species of microorganism have survived or have been reintroduced and have the ability to grow in the product; in such a case, microbial interactions can be neglected. A simple primary model which includes the lag (relevant response when considering thermally injured bacterial spores[9]) is the three phase linear model of Buchanan *et al.*[10]. A large variety of secondary models have been developed over the years[8]. However, for implementation in MRA models, often gamma-type models are preferred to polynomial models.

Recontamination is defined as the introduction of any micro-organism into the product after an inactivation step, namely post-process recontamination. Recontamination events in aseptic-UHT processes are a primary cause of product sterility failure, and therefore processes modelling these events form a key part of any

Table 1: Examples of management options applied to an aseptic-UHT-type process in a risk-based framework. See **Figure 3** for location in the process.

Contamination variation	Management Option Labels	Examples of management option
Raw material: initial contamination	MC_1	Sealing component specifications
	MC_2	Product container specifications
	MC_3	Product ingredient specifications
Microbial reduction	PC_1 and PC_2	log reduction with Peracetic acid log reduction with H_2O_2
	PrC_3, PrC_4	Choice of the packaging sterilisation agent
	PC_3	log reduction targeted during the UHT treatment
	PrC_5	Sterilisation value, F_0, targeted during the UHT treatment
	PdC_2	pH of the product
	PC_4, PC_5 and PC_6	log reduction after the cleaning procedure
Microbial increase	PrC_1	Time during which the packaging is left open on the line, empty, before sterilisation
	PrC_2, PrC_{10}	Time of intermediate storage
	PdC_1, PrC_3	pH of the product
	PrC_6, PrC_{10}	Number of valves
	PrC_7, PrC_{11}, PrC_{14}	Number of batches before running the cleaning procedure
	PC_7, PC_8, PC_9	Targeted filter efficiency expressed in log reduction
	PrC_8, PrC_9, PrC_{12}	Number of line sterilisations before changing the filters
	PrC_{14}	Time during which the container, filled with the product, is left open, i.e. time between filling and sealing
Final product	MC_4	<1 in 10,000 defective after sterility test: ✓ 6 ± 1 day at 55°C for thermophilic bacteria ✓ 10 ± 3 days at 30°C for mesophilic bacteria

aseptic-UHT MRA model[11]. An overview of recontamination models has been given in Reij *et al.*[12]. Moreover, Den Aantrekker *et al.*[13] have built a general recontamination model framework, based upon a mass-balance equation system: a contaminated source (liquid, equipment or floor) releases cells to the intermediate phase (surface, hands or air) and then cells can be transferred to the product causing recontamination.

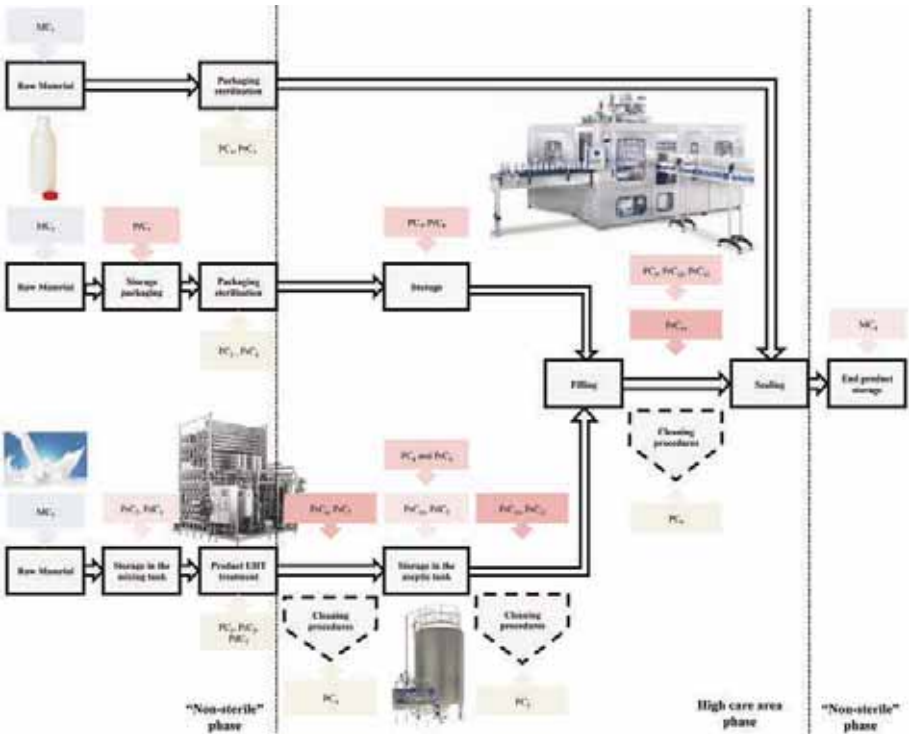

Figure 3: Aseptic-UHT food process and its possible management options (the corresponding management options are detailed in **Table 1** opposite)

Advanced probabilistic techniques

The aim of advanced probabilistic techniques is to deal with the variability and uncertainty inherent to biological processes[14]. Parameter uncertainty means uncertainty about the values of input variables, reflecting the lack of information available to estimate these values (e.g. the transfer rate parameters in the mass balance equation for recontamination). Model uncertainty also means uncertainty due to the approximation by a functional form of a real phenomenon (e.g. the recontamination by the biofilm formation). Variability differs from uncertainty: variability refers to natural or non-controlled heterogeneity between individuals within a population addressed by risk assessment (e.g. D and z values among strains of thermotolerant *B. cereus*). It is not always possible to separate entirely uncertainty and variability, although it is recommended to try as much as possible[15]. Increasingly often, probabilistic approaches (whole range of values and their probability of occurrence) are preferred to deterministic ones (single point estimate) and a large number of applications are available in the literature. However, a MRA model applied to a UHT-process line is complex; consequently it cannot be run analytically when a probabilistic approach is chosen. Therefore, numerical alternatives such as Monte Carlo simulation techniques are generally deployed. Bayesian inference is another technique used in MRA: combined to a Markov Chain technique, it enables running / solving numerically a complex model. In Bayesian inference, the natural variability is often modelled by a parametric distribution characterised by uncertain hyperparameters[16].

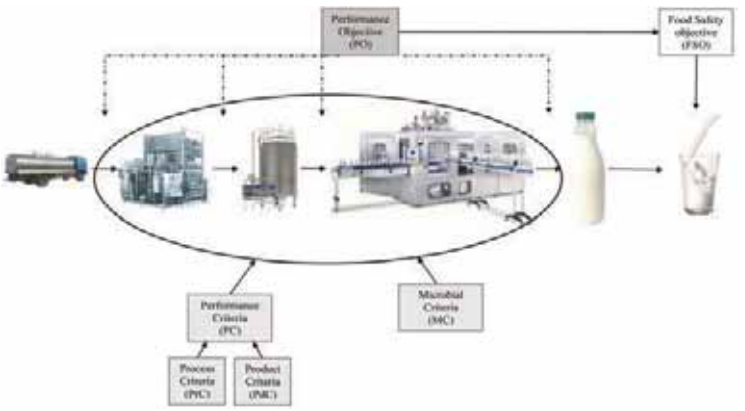

Figure 2: From the Food Safety Objective (FSO) and Performance Objective (PO) to the management options (Performance Criteria (PC), Product criteria (PdC), Process criteria (PrC) and Microbial Criteria (MC)), application to an schematic aseptic-UHT process. (Adapted from Pujol *et al*[2])

Sensitivity analysis is widely used in MRA to: i) identify the most influential variables on the model output; ii) provide a better understanding and interpretation of the analysis; and iii) identify data gaps and then prioritise future research. Sensitivity analysis might also be seen as a prerequisite for model building, to test the robustness and the relevance of the model[17]. The added value of sensitivity analysis is highlighted in case of large and complex models. As mentioned by Pujol *et al.*[2], with a MRA model applied to aseptic-UHT products, it might be difficult to identify which model modules, and inside each of these, which variables, have the greatest impact on the commercial sterility failure rate. In addition, sensitivity analysis helps in identifying and eliminating particular inputs or components of the model which are not essential, i.e. in simplifying complex models on a rational basis[15].

Risk-based framework

The food safety management is now shifting from a hazard-based approach to a risk-based approach. For pathogens, the new concepts of Appropriate Level Of Protection (ALOP) and Food Safety Objective (FSO) have emerged[18]. FSO is defined as the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of health protection. Anderson *et al.*[19] reported the historical approaches to establishing commercial sterility and presented a perspective on the establishment of a FSO for proteolytic *Clostridium botulinum*[19]. Other examples from the literature are available in Pujol *et al.*[2].

On the contrary, the link between the equivalent FSO and ALOP for a spoilage hazard has not been elaborated in the literature. As pointed out by Pujol *et al.*[2], the task of building such a 'link' is greatly aggravated by the almost infinite number of potential spoilage hazard agents capable of contaminating and growing within aseptic-UHT products. Nevertheless, the dose-response relationship which links ALOPs to FSOs

for pathogen hazards has a counterpart in the consumer complaint and sterility failure rate targeted for spoilage hazards: a targeted sterility failure rate theoretically can be related by a food company through a measure of consumer satisfaction (e.g. consumer complaint rates).

Within the risk-based food safety management metrics, the Performance Objective (PO) is directly related to the FSO but is set at a step before the time of consumption (e.g. at the point of product release at the end of the manufacture) (**Figure 2**, page 15). Modelling the effect of a heat treatment step on food product post-processed contamination and making a link with the compliance to a PO or FSO has been described in the literature[19-24]. However, often, to be operational FSOs and POs must be translated into criteria that can be controlled and measured in the food supply chain such as Performance Criteria (PC), Process Criteria (PrC) or Product Criteria (PdC) (**Figure 3**, page 15). A PC is the effect required of one or more control measure(s) working in concert to meet a PO. In an aseptic-UHT process line, the air filter efficiency could be translated to a PC[25]. Likewise, the efficiency of the complete cleaning procedure could be set as a PC to control and reduce when necessary the contamination risk. More examples of PCs are provided in **Table 1** (page 14) and illustrated in **Figure 3** (page 15). PrC and PdC are the control parameters at a step or combination of steps that can be applied to achieve a targeted reduction or the desired limitation on growth and contamination, i.e. to achieve a PC. The type of the packaging sterilisation agent in a UHT-line could be set as a PrC. The complexity of the line (pipe length, number of valves, etc.) could be expressed in quantitative terms and then translated into PrCs; the pH of the product set as a PdC; the temperature and the time of storage set as PrCs (**Table 1**, page 14 and **Figure 3**, page 15). Once defined, PrC and PdC can be translated as Critical Control Points or Operational Pre-requisite Plans in a HACCP plan[26].

Beside control measures, Microbial Criteria (MC) are commonly used in food industry. In the near future, as food safety management moves towards risk-based food management, food producers will need to provide evidence that their foods at the moment they are eaten, comply with an FSO[27]. For *C. botulinum*, an example of an FSO might be less than one product unit contaminated per 10^6 or 10^9 units[19]. Setting MC to assess such low occurrences is not realistic. However, MC might be valuable tools to assess the microbial quality (food spoilage) along the entire aseptic-UHT product process, i.e. to assess the compliance with POs set at various critical process steps: raw materials, mix blends pre-UHT, product units after filling, etc. (**Table 1**, page 14).

About the Author

Jeanne-Marie Membré has a diploma in Food Engineering and a PhD in Food Microbiology and has worked in both academia and industry. In 1989, she joined the French National Institute for Agricultural Research (INRA) of Villeneuve d'Ascq, France, where she was in charge of the predictive microbiology research programme. From 2003 to 2009, she worked at the Safety & Environmental Assurance Centre of Unilever, in Bedford, UK, where she developed predictive models and exposure assessment models for a wide range of food applications. Since 2010, she has been working in INRA of Nantes, France. Jeanne-Marie is currently the leader of the Microbiological Risk Assessment in Food Group at the UMR1014 Secalim. She is involved in a large number of research projects but also in educational programme such as the Erasmus Intensive Programme Predictive Modelling and Risk Assessment. Her experience encompasses predictive microbiology, microbial risk assessment, applied statistics and food safety. Jeanne-Marie has published more than 50 articles in peer-reviewed journals, is a member of the International Association of Food Protection, belongs to the scientific board of Journal of Food Protection and is expert at ILSI-Europe.

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Listeria monocytogenes: what have we learned from the last 30 years?

Listeriosis is a foodborne disease caused by the facultative intracellular pathogen *Listeria monocytogenes*. The bacterium was first described in 1926 in laboratory rabbit zoonosis by Everitt G.D. Murray, and remained underreported for almost 60 years. It used to be considered as a scientific laboratory tool for cellular motility mechanisms, until 1981 when it was reported for the first time in a foodborne outbreak in coleslaw (Nova Scotia, Canada, 1981).

In the past 30 years, *L. monocytogenes* has been recognised as a major foodborne pathogen, responsible for fewer infections than *Salmonella* spp. or *Campylobacter* spp., but with the highest hospitalisation rate (90 per cent), as well as high mortality rate. Approximately 2,500 illnesses and 500 deaths are attributed to listeriosis in the United States annually. In Europe, the rate of listeriosis ranges from two to six cases per million, depending on the reporting countries and it is still increasing mainly with the elderly and immunocompromised people. Although many tests are done by the food industry to search for the bacteria in food samples, a significant increase has been observed in developing countries since 2003.

Since the coleslaw outbreak of 1981, *L. monocytogenes* is considered to be one of the major foodborne pathogens, not because of a high prevalence in foods, but due to the high morbidity / mortality of the disease listeriosis that it can cause[7].

Following the risk assessment performed by The Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) on *L. monocytogenes*[9], guidelines were published by the Codex Committee on Food Hygiene and defining microbiological criteria in Ready to Eat (RTE) foods depending on the growth potential of *L. monocytogenes*[2].

Following the publication of these guidelines, Codex member states have updated their regulations accordingly[4,12]. Health Canada's recently published guidelines[6] are aligned on the proposed rationale, while the USDA-FDA published another approach of 'zero tolerance' of *L. monocytogenes* in RTE foods[14], although the classification versus growth potential was considered in their prior risk assessment[13].

The rise of a pathogen

As listeriosis is now recognised to be almost exclusively foodborne, as stated by the risk assessment approach proposed by ILSI[7] and the World Health Organization[9], industrial food manufacturers must exhibit: "good manufacturing practices, sanitation standard operating procedures, and hazard analysis critical control point programs to minimise environmental *L. monocytogenes* contamination and to prevent cross contamination in processing plants and at retail."

Listeria being a ubiquitous microorganism, safety of the end-product cannot be guaranteed solely by the analysis of food samples, but also

requires a specific assessment of environmental contamination. Results of environmental samplings of *Listeria* spp. and *L. monocytogenes* over the last 25 years have taught two main lessons to the food industry: firstly, the prevalence of the *Listeria* genus is higher than the monocytogenes species alone, *L. innocua* having the highest occurrence. And secondly, that *L. monocytogenes* exhibits a high interspecies variability, with persistent strains which can stay in a plant for years, and transient strains which do not colonise the environment.

Molecular typing is a very good tool for tracking the contamination pathways of *L. monocytogenes* at a plant. But not all food industry manufacturers can go through this costly inspection[3].

Searching for the whole genus gives higher efficiency at a lower cost, or even a gain of time depending on the method chosen. *L. innocua,* considered to be the genetic ancestor of the genus, is a better ecological competitor than *L. monocytogenes* ½ (a, b or c) and 4b. During the analytical enrichment procedure, the growth of *L. innocua* can overcome the growth of *L. monocytogenes*, leading to a false negative if one is only searching for the monocytogenes species. Depending to the type of food industry, the *innocua* versus *monocytogenes* ratio can vary from one to the third to one to the tenth. Searching for *Listeria* spp. can better confirm the relevance of zoning and the critical points identified by HACCP plan, in order to prevent contamination with *L. monocytogenes*, therefore giving better food safety assurance to the food business operator.

Taxonomy

The genus Listeria currently comprises 10 species: *L. fleischmannii, L. grayi, L. innocua, L. ivanovii, L. marthii, L. monocytogenes, L. rocourtiae, L. seeligeri, L. weihenstephanensis* and *L. welshimeri*.

Cases of human listeriosis are almost exclusively caused by the species *L. monocytogenes*. Although some rare cases in patients with underlying diseases have been reported, *L. ivanovii* and *L. seeligeri* are not considered to be a foodborne hazard.

Ecology of *Listeria* spp.

L. monocytogenes tolerates harsh conditions and may therefore survive or grow in different types of foods. The organism can grow in low temperatures (-1.5 to 45°C) and in a wide pH range (4.3 to 9.1). It can also grow in salt concentrations of up to 10 to 14 per cent and tolerates low water activity (Table 1).

Table 1: Commonly accepted cardinal values of growth characteristic of *L. monocytogenes*

	Min.	Opt.	Max.
Temperature (°C)	- 0.4	30-37	45
pH	4.0	7.1	9.6
a_w	0.90 (glycerol)	/	/
	0.92 (NaCl)	/	/
	0.93 (Saccharose)	/	/

L. monocytogenes can be found in a large variety of habitats including soil, vegetation, silage, sewage, water and faeces of healthy animals and humans. It is frequently present in foods of animal and plant origin and can become endemic in food processing environments. It may also be present in cooked foods as a result of post-process contamination or inadequate heat treatment[8,10].

Pathogenesis of *L. monocytogenes*

Listeria invasive diseases have been described in more than 40 animal species. The mechanism is very conservative and generally leads to abortion, septicaemia, meningitis and encephalitis, as well as some reported cases of diarrhoea, skin infections and endocarditis. Thirteen serotypes have been identified for *L. monocytogenes*. All of these may be associated with human listeriosis; however, most human infection is associated with the serotypes 1/2a, 1/2b or 4b, with a hospitalisation rate of over 90 per cent and a death rate of 20 – 30 per cent of those infected[5,11].

Two types of disease are associated with *L. monocytogenes*: non-invasive or invasive listeriosis. Non-invasive listeriosis (referred to as febrile listerial gastroenteritis) is the milder form of the disease. Symptoms include diarrhoea, fever, headache and myalgia (muscle pain). Symptoms occur after a short incubation period. Outbreaks of this

disease have generally involved the ingestion of high doses of *L. monocytogenes* by otherwise healthy individuals.

Immunocompromised persons, the elderly, pregnant women and neonates have the highest risk of infection because of their weakened immune system. This disease is characterised not only by the severity of the symptoms but also by a high mortality rate.

Healthy adults and children occasionally get infected with *L. monocytogenes*, but they rarely become seriously ill. Individuals whose cell-mediated immunity is suppressed are more susceptible to the devastating effects of listeriosis. Pregnant women naturally have a depressed cell-mediated immune system and in addition, the systems of foeti and newborns are very immature and are extremely susceptible to these types of infections. Other adults, especially transplant recipients and lymphoma patients, are given necessary therapies with the specific intent of depressing T-cells, and these individuals become especially susceptible to *L. monocytogenes* as well.

Prevalence in food products and food business premises

L. monocytogenes can be found in a wide variety of raw and processed foods. Milk and dairy products, various meats and meat products such as beef, pork, fermented sausages, fresh produce such as radishes, cabbage, seafood and fish products have all been associated with *Listeria* contamination (see Table 2 for major reported outbreaks).

Listeria spp. and *L. monocytogenes* have been isolated from a variety of sites, although these bacteria are most frequently found in moist environments or areas with condensation or standing water or food residues, including drains, floors, coolers, conveyors and case washing areas. Biofilms serve as a source of *L. monocytogenes* for processed foods[1,15].

Safety of the end-product cannot be guaranteed solely by the analysis of food samples but by verifying that proper control measures are in place. This requires a specific environment and process line monitoring as suggested in EU Regulation 2073/2005 (Article 5: Food business operators manufacturing RTE foods, which may pose a *L. monocytogenes* risk for public health, shall sample the processing areas and equipment for L. monocytogenes as part of their sampling scheme).

Control measures to mitigate the risk

Historically, heat treatment is applied to raw materials to eliminate the initial microbial contamination. The current treatments are based on either industry guidance, codes of practice or regulation based mostly on a historical approach depending on the population reduction required.

- An overall recognised pasteurisation treatment of raw milk for 15 seconds at 75°C would result in elimination (6-log reduction) of *L. monocytogenes*.
- The pasteurisation of cooked chilled foods for a minimum of two minutes at 70°C would result in at least a 6-log reduction of the organism.

Growth of *L. monocytogenes* in RTE food can be inhibited using one or more of the following control measures:

- pH less than or equal to 4.0 (or 4.4 in case of lactic acid as main organic acid).
- Water activity less than or equal to 0.92
- Formulation containing one or more inhibitory substances that, alone or in combination, prevent the growth of *L. monocytogenes* (e.g. biopreservation with food starters)

Table 2: Major reported food borne outbreak of *L. monocytogenes*

Product Category	Vehicle	Country	Year	Number of cases (deaths)
	Coleslaw	Canada	1981	41 (18)
Vegetables	Sweetcorn (salad)	Italy	1997	1566; 292 hospitalisations
	Cantaloupe	USA	2011	146 (30)
	Pasteurised milk	USA	1983	49 (14)
	Cheese	USA	1985	142 (48)
	"Vacherin" soft cheese	Switzerland	1983-1987	122 (34)
	Brie de Meaux	France	1995	20
	"Livarot" and "Pont l'Evêque" soft cheeses	France	1997	15
Milk Products	Butter	Finland	1999	25 (6)
	Cheese	Canada	2002	42
	Pasteurised Cheese	Canada	2008	38 (5)
	Quark Cheese	Austria	2010	14 (4)
	Ricotta	USA	2012	14 (4)
	Cheese	USA	2013	6 (1)
	Pâté	UK	1987-1989	>350
	Jellied pig's tongue	France	1992	279 (63), 22 miscarriage
	Rillettes	France	1993	38
	"Gravad"	Sweden	1994-1995	9
Meat and Fish Products	Hot-dog, "deli meat"	USA	1998-1999	101
	Rillettes	France	2000	6
	Turkey and chicken-based ready-to-eat products	USA	2000-2001	29 (4)
	Sliced, cooked turkey	USA	2002	46 (7)
	Meat	Canada	2008	57 (22)

- Strict maintenance of cold chain
- Prevention of cross-contamination (GMP) and re-contamination (GHP) of heat treated food products.

Non-food contact surfaces (FCS) contamination with *Listeria* spp., including *L. monocytogenes*, usually precedes FCS contamination.

Testing for *Listeria* spp. and reacting to positive results as if they were *L. monocytogenes* provides for a more sensitive and broader verification and control program, than would testing for *L. monocytogenes* alone, most particularly considering the expected very low prevalence of this pathogen in the food processing environment.

While the environmental monitoring program in dry area is focused on *Salmonella* spp. with Enterobacteriaceae as hygiene indicators, the monitoring of *Listeria* spp. should be focused on the wet zones of the environment, e.g. the cleaning station, water condensates, and drains if any[1,15].

Regulatory status

Different microbiological criteria apply considering the categories of food products:

- RTE foods in which growth of *L. monocytogenes* will not occur
- RTE foods in which growth of *L. monocytogenes* can occur.

For RTE foods in which growth will not occur, rejection level is set at 100 colony-forming units per gram of the products in five samples. In RTE foods in which growth can occur, absence in 5x25-grams product samples is required.

Countries differ in their regulatory approach to the presence of *L. monocytogenes* in RTE food. In the European Union, Commission Regulation (EC) No 2073/2005 sets limits similar to Codex Alimentarius guidelines, with complementary introduction of criterion (absence in 10x25-grams product samples) for infant products and RTE foods for special medical purposes. In the United States, the United States Department for Agriculture maintains a policy of 'zero-tolerance' for *L. monocytogenes* in RTE foods[14]. In Canada, an update of the 'Policy on *L. monocytogenes* in RTE foods' has been completed in 2011 and is aligned on the European approach[6].

Conclusion

Despite its very low occurrence in food products and limited number of infection reported, *L. monocytogenes* remains a key pathogen of concern for the food industry, due to its high mortality rate and the target populations (infants, pregnant women, elderly and the immuno-compromised). Due to its ubiquity in the environment and its high ability of colonisation of food industry premises, the strict application of GMPs / GHPs and continuous surveillance through the environmental monitoring program are the most efficient hurdles to ensure a safe food supply vesus this microbial hazard.

More generally, considering *L. monocytogenes* or other relevant foodborne pathogen, the quality and safety of foods is most efficiently assured by the design and implementation of appropriate control measures, i.e. GMP/GHP and HACCP as applied during production and throughout the food chain.

This is a much better approach than sole microbiological testing of finished products or ingredients, which is of limited value to assess their safety, yet necessary at least for regulatory purposes and to prove the proper implementation of the food safety management control measures.

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Further reading

- WHO Listeria monocytogenes risk assessment (MRA Series 4 & 5) http://www.who.int/foodsafety/micro/jemra/assessment/listeria/en/
- Codex Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Ready-to-Eat Foods (CAC/GL 61-2007) http://www.codexalimentarius.net/download/standards/10740/CXG_061e.pdf
- Achieving Continuous Improvement in Reductions in Foodborne Listeriosis—A Risk-Based Approach. ILSI research foundation / risk science institute, expert panel on Listeria monocytogenes in foods. Journal of Food Protection, Vol. 68, No. 9, 2005, Pages 1932–1994
- http://www.ncbi.nlm.nih.gov/pubmed/16161698
- Center for Disease Control and Prevention – United States. Listeria (Listeriosis) http://www.cdc.gov/listeria/
- European Food Safety Authority. Listeria http://www.efsa.europa.eu/en/topics/topic/listeria.htm

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The need for risk assessment and validation in frozen food manufacturing

Frozen foods are relatively fresher and safer than produce which comes out of the farm directly or those that were subjected to extreme processing to make shelf-stable and safe. However, freezing does not inactivate microorganisms in food, it only slows down growth. The freezing process is used mainly to preserve foods for a longer period of time. Frozen products still can harbour microorganisms which may become a food safety concern if not properly handled by manufacturers, retailers and consumers. Prepared frozen foods must be safe prior to freezing in order to be safe after freezing.

Outbreaks through frozen foods have been reported occasionally though not to the extent of outbreaks reported from other food categories. Microbial growth at temperatures below 0°C is more likely to be that of yeast and moulds rather than bacteria. While frozen foods typically have fewer food safety concerns, manufacturers and food processors still need to make sure the products are manufactured with food safety in mind. This becomes even more important today as consumers look for foods with minimally processed, natural foods with less or no preservatives.

Food safety professionals cannot remain complacent as public health can never be compromised and evaluated based on outbreak levels. This is especially important for food manufacturers as they put many of their interests in line including regulatory compliance, brand integrity, and costs associated with product recalls. From the regulatory

perspective, little emphasis was placed on frozen foods because of their positive historical record. However, with so many products flooding the market from manufacturers ranging from big corporations to fresh entrants, it is becoming increasing challenging for regulatory agencies to enforce their safety standards. Changing consumer behaviour complicates this further as they look for products that are minimally processed, processed using novel technologies, ethnic cuisines, different cooking methods, and all sort of innovative products at no additional cost to them. Thanks to advances in temperature measurement, refrigeration and freezing, food manufacturers are mostly able to cope with public demand with innovations in product development and packaging. However, there are still many challenges that remain unresolved due to the complexity involved in the frozen food business. There are many areas in the product lifecycle that can go wrong, resulting in risk to public health.

This article focuses on areas of concern related to frozen food safety and the need for risk assessment and challenges in validation processes in some frozen foods manufacturing. For the sake of simplicity, risk areas associated with frozen food manufacturing can be classified into:

- Ingredient procurement
- Product preparation
- Process control
- Packaging
- Storage, transport and distribution.

Ingredient procurement

The final quality of the frozen foods is greatly influenced by the quality of ingredients. Almost all of the food manufacturers count on the guarantee of the ingredient suppliers for the safety of their ingredients. The certificate of analysis (COA) furnished by the ingredient manufacturers represents only a snapshot of an incoming lot and it should not be considered sufficient. Many food manufacturers lack the resources, money and time to evaluate the incoming raw ingredients to ensure safety levels. In lieu of this, food manufacturers need to place stringent controls for qualifying vendors by conducting periodic audits of their food safety processes and systems.

Cost reduction measures involve changing the ingredients and vendors more frequently than before, resulting in less time for quality control. Temperature abuse during various stages of frozen ingredients manufacturing and shipping is a big cause of concern as very little control is possible, especially with products like meat protein and egg products. An option of having more than one supplier with enough control limits on each of the product parameters will help ensure a constant supply with no compromise on quality and safety. Ensuring the safety of ingredients procured across borders is a daunting task for both food manufacturers and regulatory agencies. Global Harmonization of food safety regulations has a long way to go to make this process easier for the benefit of everyone.

Product preparation

Most ingredients used in the manufacture of frozen products will be used in a refrigerated or cold state prior to assembly and the freezing process. Biological hazards of concern for frozen products will be based on the initial microbial load that could be introduced either from other raw ingredients and/or processing environment such as equipment, personnel, or even the water itself.

The effect of freezing on microorganisms depends on the type of food, the rate of freezing, the kind of microorganisms, and freezing temperature[1]. Potential sources of contamination need to be identified very carefully and measures need to be taken with appropriate limits and control procedures.

Quality parameters like pH, acidity, salt and water activity which have a profound influence on safety need to be clearly set and maintained. While controlling all these factors could be achieved with a diligently developed food safety program, training of employees with GMPs, allergen control, and food safety issues is essential to effectively implement it. Clear accountability and rewarding of responsible behaviour should be encouraged.

Processing line

The use of pre-cooling process for some ingredients used in frozen products is to lower the heat from one temperature to another and facilitate the freezing process. Products that are pre-cooled prior to freezing must be monitored under a restricted time frame and strict hygiene conditions to avoid any potential microbial outgrowth. All ingredients are recommended to be used or further processed as quickly as possible prior to freezing, to avoid the temperature danger zone between 135°F (57°C) to 70°F (21.1°C) for potential microbial outgrowth (FDA Food Code, 2009).

It is recommended that once frozen products achieve the frozen state, they are kept frozen. The practice of holding and thawing frozen products for too long may provide the right environment for microorganisms to grow. For this reason, monitoring of the frozen process and equipment is critical to ensure the control of

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biological hazards. The monitoring controls will depend on the type of product, equipment, freezing process and complexity of manufacturing operations. Some examples of monitoring controls during the freezing process include: freezer controls and settings with temperature limits; load for each cycle, formula / recipe to ensure no changes on ingredients or set limits that can affect the freezing process; storage temperature of finished product temperature (recommended -18°C); and freezer maintenance and capabilities. Frozen products shall be released for distribution only when they had been frozen to -18°C.

Another two key steps during process control include proper recordkeeping and calibration which must be carefully reviewed to ensure the freezing process was followed as designed. The use of calibrated equipment such as thermometers is a critical step during freezing and it is recommended to verify calibration equipment at least once at the beginning of each production shift.

Process control plays a major role in risk alleviation. Most of the contamination issues in the previous steps can be controlled with a carefully controlled process line. However, unclean process equipment and inadequate thermal processing step may pose a bigger safety threat and was implicated in a number of outbreaks. Quality of air and environment in the manufacturing plant needs to be carefully monitored with swap testing as frequently as possible. Unattended minor repairs and unhygienic design of product flow (e.g. slope of pipes) will escape many of the auditors' watchful eyes. Inadequate cleaning and sterilising of the fillers and filler area is a risk area acknowledged by everyone. The freezing equipment and medium must not act as a source of re-contamination and must be included in a master sanitation plan. All corrective actions should be documented, and investigated to prevent reoccurrence of deviation.

Packaging

Packaging is a very risky food safety area which is often neglected or paid little attention in the risk management process. Post-process recontamination mostly results in spoilage and the possibility of pathogenic infection cannot be ignored. Packaging material of the final container (films, trays, lids) and intermediate storage totes, drums, etc. are sources of recontamination of the processed products. All types of packaging must be properly inspected, stored and managed by the factory to ensure safety and quality.

Other issues such as improperly ventilated package designs and aseptic wrapping of foods may lead to anaerobic conditions and pose a bigger threat. Intentional sabotage and tampering with the finished food product is a big concern in this new world and need to be avoided with tamper evident packaging designs. Physical contamination with chipped glasses, delaminated films, and broken plastics can result in extensive recalls and damage the brand of the manufacturer apart from hurting the consumer psyche. Installation and periodic validation of metal detectors and visual inspection systems like x-rays that will detect foreign objects to the level of required specifications and safety has become mandatory in many food processing plants. Some of the package designs that may not be a food safety concern but may be of consumer safety concern include, steam venting valves, deshaping and fraying of trays, insufficient flanges, temperature masking, etc.

Storage, transport and distribution

The dangerous zone for keeping the products and ingredients is 40-140°F and this needs to be completely avoided. Coolers and freezers should have enough capacity and be efficient enough to cool the product below this dangerous threshold level rapidly. Even holding the product at a higher temperature for a longer time (due to a failed subsequent operation) is a risk for thermophilic spoilage. A clear case of cross-contamination is the reported incidence of contamination in pasteurised ice cream mix that was shipped in the same container that was used for transporting unpasteurised egg. This resulted in *Salmonella* Enteritidis infection in thousands of people[2].

There is a need for public education and stringent permit approvals for transportation of refrigerated and frozen foods. Temperature abuse, insufficient sanitation of transport vehicles, improper handling of product packages can bring economic and brand loss to the food manufacturer[3]. Controls must exist to inspect any truck delivering frozen ingredients. These trucks should be inspected in the receiving dock areas prior to receiving any frozen materials and the units must be capable of holding food below 0°C.

Ice used for distribution could be a source of recontamination if the packaging seals are not intact (FDA Food Code, 2009). Temperature control at distribution centres and grocery display cabinets are considered inefficient by various study reports. Not properly maintaining the storage temperature will help microorganisms to grow exponentially especially when there is little or no competition for available nutrients in the processed products.

Risk assessment is not limited to these major areas of food manufacturing. Personnel with expertise in various stages of

food manufacturing (farm to fork) should be involved in making a sound judgment on the potential risk areas and the control measure to eliminate them.

Validation needs

Parameters to be considered when validating the freezing process include the type of freezing equipment, amount of product, and if the product is pre-packed, the type of packaging. The efficiency of the freezing process will be dependent upon the initial product temperature, product size, shape and free water content[4].

There is much equipment used in the food production process which will have a profound effect on the safety of the finished products. There are a number of heating and cooling devices that are used for a variety of reasons. Accurate calibration and effective use of them by trained personnel are necessary to avoid any insufficiency in the applied lethality. Even with calibrations, which are spaced over a period of time, it should be mandated that this equipment is validated for the delivery of intended heating or cooling.

With a recent survey indicating that a majority of consumers strongly believing that food manufacturers are responsible for the safety of the products they buy, the onus is now solely on the food business entities. Hence the need for food industries to conduct food safety risk assessment for each of their products and validate each of their control measures scientifically. Generating relevant scientific data by professionals with food safety expertise increases the comfort of public and government regulatory agencies. In the US, the implementation of the Food Safety Modernization Act (FSMA) demands proper maintenance of evidences by food manufacturers on their validation process along with their GMP and HACCP plans. As the frozen foods industry continues to grow, the focus on risk assessment and potential hazard vehicles will increase among the scientific community. Major industrial challenges like business sustainability and continued growth, minimising product recall, brand maintenance can very well be accomplished with greater emphasis on food safety risk assessment. There is also a need for the industry to work together with regulatory agencies and academia to effectively implement food safety control measures.

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A novel approach to reducing the total and saturated fat content of baked goods

In 2008, the Food and Agricultural Organisation and the World Health Organisation reviewed their recommendations on dietary fat and fatty acids in view of the growing evidence on dietary fatty acids and health outcomes[1]. The key recommendation was that our diet type should change to limit the saturated fatty acid content to 10 per cent of the dietary energy intake. The saturated fatty acids should be replaced with polyunsaturated fatty acids. By adjusting the ratio of saturated to unsaturated fatty acids in the diet, the level of low density lipoprotein cholesterol in blood serum is reduced, which correlates with a decrease in the risk of coronary heart disease.

Fat, in either saturated or unsaturated form, is the most calorie dense nutrient available to us. The public health responsibility deal, which the UK Food Industry has signed up for, was put in place following the Call to Action on Obesity in England[2], which set out the importance of action on obesity, and issued a challenge to the population to reduce its total calorie consumption by five million calories (kcal) a day. One route to do this is to reduce total fat content. Additionally, the public health responsibility deal saturated fat reduction pledge[3] recognises the need to reduce saturated fat consumption to less than 11 per cent of food energy compared to current levels of 12.7 per cent.

Subsequently, there are drivers to reduce the total fat and also the saturated fat content of our diet. Bakery products such as cakes and biscuits contain significant amounts of fat. For example, the short dough biscuit recipe used in this study has a total fat content of about 22 per cent, 41 per cent of which is saturated fat, and the standard yellow

or high ratio cake recipe has a total fat content of about 16 per cent, 35 per cent of which is saturated fat.

Fat has a range of technical roles in both cake and biscuit manufacture and any fat reduction or replacement with an alternative material must ensure that these roles are still met.

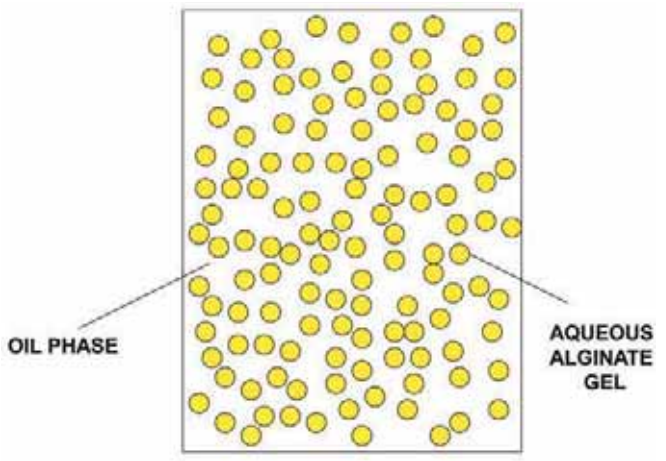

Figure 1: Schematic of alginate gel-in-oil emulsion

The key role of fat in biscuit dough has been described as shortening[4], which gives biscuits their characteristic 'melt in the mouth' crumbly texture. The mechanism by which fat contributes this property to biscuits is by coating flour particles during the mixing of biscuit dough. This fat coating prevents gluten from absorbing water during mixing and becoming elastic and extensible[5]. Gluten development is essential in bread dough but is to be avoided in biscuit dough; otherwise the biscuits produced can be undesirably tough and chewy.

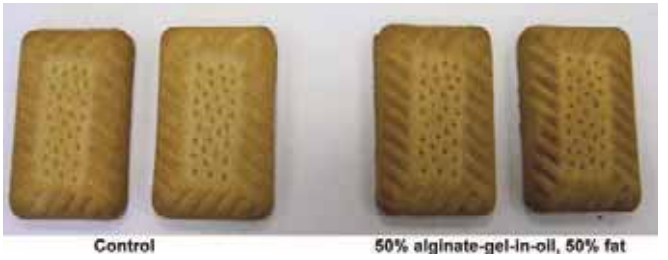

Figure 2: Biscuits containing 100% fat and 50% fat

A secondary role of fat is to coat any air bubbles present in order to enhance their stability during baking. This role is similar to the stabilising effects of fats on bubbles in bread dough and cake batter[6,7] and prevents the bubbles rupturing and coalescing into larger bubbles.

While both liquid and solid fat can be used to coat wheat particles to restrict gluten development, the firmness, and therefore the machine-ability of dough, is directly related to the solid fat index (SFI) of the dough fat[8]. Dough which contains liquid oil at the point of handling is difficult to work and develop[9]. The ability to coat bubbles to retain integrity during dough handling and baking is also best found in a fat with a developed crystalline structure rather than in a liquid oil[10]. Hard fats, rather than oils, are used to provide the required functionality in biscuit dough, with the naturally produced ingredients of choice being either palm oil, which has a melting point of around 36°C, or butter, which melts in the temperature range 32 – 35°C.

The use of saturated fats is therefore central to biscuit technology but also has a major role in cakes. By interfering with gluten development, fat contributes to a shorter texture with a softer eating crumb. Fat also acts to improve the shelf life of cakes since it acts as a moisture barrier, preventing significant increases or decreases in moisture content over typical storage conditions. Fat provides intrinsic flavour and lubricates the bolus of cake and biscuit during chewing and swallowing. Fat is also a reservoir for fat-soluble flavour components generated during baking.

Figure 3: Control cakes compared to cakes where 50 and 25 per cent of the fat has been substituted with a 50:50 alginate gel-in-oil emulsion

Replacement or reduction of fat in both biscuit and cake therefore requires any alternative material to perform a range of technical functions associated with the development of structure and texture together with eating quality considerations. Having reviewed the literature on fat reduction, it was decided to pursue an approach based on modified water-in-oil emulsions.

The approach adapted was based on the teaching of patent WO 93/19613[11], which relates to the use of a gelling system to replace water in margarine or reduced fat spreads. A liquid system containing all the necessary elements to form a thermally stable gel is prepared. The liquid system is then emulsified in oil to produce a water-in-oil emulsion. The liquid-to-solid transition is triggered once the emulsion is formed to give a gelled aqueous phase. The gelled aqueous phase is thermally irreversible, which means that the aqueous phase of the margarine or reduced fat spread produced has rheological properties which are largely independent of temperature. In practice, the liquid system can be a sequestered sodium alginate or sodium pectate solution containing a dispersed sparingly soluble calcium salt. Alginate and pectate form gels in the presence of calcium. The level and

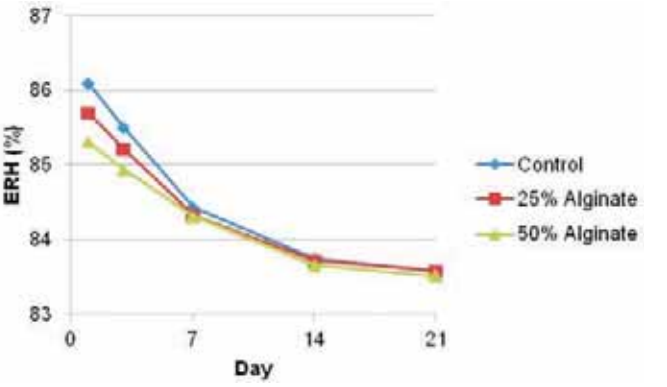

Figure 4: ERH changes over 21 days for cakes (measured at 21°C)

type of sequestrant can be used to control the rate of release of calcium such that gelation happens after the emulsion is formed. This is shown schematically in Figure 1 (page 29).

Figure 2 (page 29) shows examples of control and test biscuits where the reduced fat biscuits had 50 per cent of the shortening replaced by a 50:50 alginate gel-in-oil emulsion prepared using this technology. The corresponding dimensional measurements, hardness values and moisture contents are shown in Table 1.

The reduced fat biscuits had lower moisture contents than the control and were shorter in length than the control biscuits. The stack height and the width of the sets of biscuits were similar. The control biscuits were harder than the reduced fat biscuits; however, this difference was not significant.

The control biscuits had a total fat content of 21.6 per cent and a saturated fat content of 10 per cent. The test biscuits showed a decrease of 21 per cent in total fat and a significant decrease of 41 per cent in saturated fat compared to the control, enabling a claim of 'a reduction in saturated fat content' or 'reduced saturated fat' to be made.

This approach was also evaluated in high ratio cakes. Figure 3 (page 29) shows examples of control (full fat) cakes compared to cakes where 50 per cent and 25 per cent of the fat has been substituted with a 50:50 alginate gel-in-oil emulsion.

It can be seen that the appearance and volume of test and control cakes are similar.

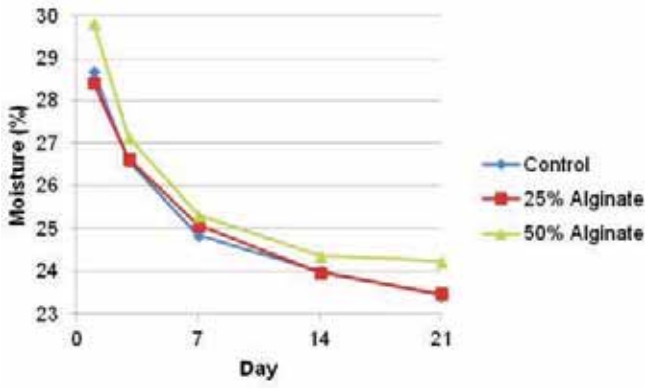

Figure 5: Moisture changes over 21 days for cakes (measured at 21°C)

Water activity (ERH) and moisture content were measured over 21 days. The ERH values are shown in Figure 4 (page 29) with the corresponding moisture contents shown in Figure 5.

It can be seen that both moisture content and ERH of cakes decreased over storage. There is some evidence to suggest that the ERH of cakes made with the alginate gel was lower than comparable control cakes, despite having higher moisture contents. This suggested that the encapsulated water added through the alginate gel was less mobile than the water added to the conventional recipe. This means that a moist eating cake with a reduced fat content can be made with no reduction of mould-free shelf life. The fat composition of the cakes is shown in Table 2.

Table 1: Biscuit measurements for reduced fat biscuits compared to control

Sample	Stack Height (mm)	Length (mm)	Width (mm)	Moisture Content (%)	Hardness (g)
Control	100	6.7	4.1	3.4 ± 0.0	2910 ± 407
50% fat, 50% 50/50 alginate gel-in-oil	102	6.6	4.1	3.2 ± 0.0	2698 ± 909

Table 2: Cake fat content

Fat Type	Control (g/100g)	50% shortening reduction	Change (%)	25% shortening reduction	Change (%)
Total fat	16.4	13.8	(15.8)	15.2	(7.3)
Saturated fat	5.8	3.9	(32.8)	4.8	(17.2)
Mono-unsaturated	7.1	5.2	(26.8)	6.2	(12.7)
Poly-unsaturated	2.8	4.1	46.4	3.5	25.0
Trans fatty acids	<0.1	<0.1	0	<0.1	0

Figures in brackets are negative values showing a fat reduction. The cake containing alginate gel-in-oil emulsion with a 50 per cent shortening reduction has a saturated fat reduction of 32.8 per cent, again enabling a claim of 'a reduction in saturated fat content' or 'reduced saturated fat' to be made.

In conclusion, the use of alginate gel-in-oil emulsions offers food manufacturers a means of achieving total fat reduction and also significant saturated fat reduction in both short dough biscuits and high ratio cakes with no compromise on product quality. This novel approach to reformulation can help manufacturers work towards the targets of the public health responsibility deal, and the saturated fat reduction pledge in particular.

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8 – 10 April 2014
Baltimore, Maryland, USA

Solutions for today, planning for tomorrow

The 16th Annual Food Safety Summit Conference and Expo; the largest solutions-based conference and expo for the food industry in North America, explores the most critical food safety issues facing the entire food industry.

Held from 8 – 10 April 2014 at the Baltimore, Maryland Convention Center, this year's event will feature a line-up of extensive educational seminars, high level industry and government speakers, workshops, networking events and a large exhibition floor. Through hands-on workshops, specialised certification courses, in-depth educational sessions and a resource-rich Exhibit Hall, attendees will find practical, real-world solutions to their food safety challenges.

"The Food Safety Summit provides solutions for today, planning for tomorrow, with a carefully developed education programme designed to address the critical issues facing food producers, processors, retailers, distributors, and regulators," said Scott Wolters, Event Director for the Food Safety Summit. "Our goal is to advance food safety by providing relevant food safety information and solutions across the entire food supply chain, while keeping the industry, businesses and consumers safe. This year the Educational Advisory Board has developed a stellar programme addressing some of the most pressing concerns while providing the most relevant solutions."

The event will kick-off on Tuesday 8 April with three half-day morning workshops featuring: A One-Health Approach to Improving Food Safety; Pre-Requisite Programs: The Building Blocks for a Successful Safety & Quality Management Strategy; and Traceability: Taking a Mock Recall to the Next Level. In the afternoon there will be a half-day workshop specifically focused on Meeting Consumer and Food Safety Modernization Act (FSMA) Food Safety Expectations.

The Food Safety Summit 2014 keynote session, which will take place on Wednesday 9 April, will feature Ed Lonergan, CEO of Chiquita Brands, and Don Zietlow, CEO of Kwik Trip, Inc. During this unique presentation, Lonergan and Zietlow will share their passion and commitment to food safety and provide insights on how they work with their food safety professionals to ensure the safest, highest quality products are reaching their customers and consumers.

"Our Educational Advisory Board was instrumental in developing the concept for this keynote presentation as a way to educate our audience of food safety and quality control professionals on how best to work with the C-Suite within their organisation," commented Wolters. "We are also excited to be offering a follow-up session which will feature the leaders of food safety for Chiquita Brands and Kwik Trip as well as several other companies who will share their success in obtaining senior management commitment to food safety."

Following the keynote, attendees who heard from CEOs during the morning presentation will have the opportunity to join Dr. Jay L.E. Ellingson, Corporate Director of Food Safety and Quality, Kwik Trip, Inc. and Courtney Parker, Vice President Quality & Food Safety at Chiquita Brands as well as the heads of food safety for other companies and learn how to work more effectively with the C-Suite/Senior Management at a Food Company. This panel of leading food safety professionals will share their top five strategies on how they earned and maintained a strong relationship with their CEOs and others in the C-Suite.

In addition, the Food Safety Summit will also offer 20 in-depth education sessions on new and noteworthy topics including: Food Fraud / Economically Motivated Adulteration; Food Safety Hazards; Storage and Distribution; Preparing for and Responding to Natural Disasters; Management Commitment to Food Safety; Hot Topic and Leading Issues; Food Safety for Foodservice Operators and Retailers; and more.

After the success of last year's Town Hall discussion, the FDA's Michael Taylor has once again been invited to the Summit and joining him will be USDA's Brian Ronholm, and AFDO's Joseph Corby to participate in an open forum, where they will address the most pressing issues for today and tomorrow in regards to regulatory agencies and the private sector. Questions and answers with the audience will be encouraged.

For fururther information:
www.foodsafetysummit.com

Webinar

Date: Thursday 10 April 2014
Time: 15:00 BST / 16:00 CET / 10:00 EDT

Food contamination an issue? – Discover solutions for your everyday sample preparation needs

Synopsis:

As food quality and food integrity has become a major topic for the consumer, the penetration path of contaminants in our food chain deriving from the food production and/or the food packaging and storage conditions is gaining attention. Fast and reliable ways of sample preparation and analysis are discussed showing up-to-date examples and how the regulations are applied.

This webinar focuses on new methods and applications in food contamination analysis, bringing together some of the leading experts in food contact materials (FCM) and provides an interactive learning environment for scientists working in this field.

What will you gain from this webinar?

- You will be provided with tangible ways to streamline your sample preparation to detect mineral oil hydrocarbons in cardboard and food as well as Bisphenol A in canned food
- BUCHI´s solutions for these applications are presented using examples from industry
- Furthermore, you will learn about health and safety risks in the field of food packaging and storage. This will be linked to up-to-date information from the leading regulatory authorities.

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Speakers:

Sabrina Moret
Associate Professor, Department of Food Science, University of Udine

Duncan Goodwin
Director of Technical Services, Supply Chain Assurance, NSF

Susanne Feifel
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Register now at: www.newfoodmagazine.com/foodcontamination

Martine Alles
Director Developmental Physiology & Nutrition and Simone Eussen, Nutrition Scientist, Nutricia Research

The relevance of reducing sugar and sweetness in products intended for infants and young children

The early years of life are a period of very rapid growth and development. Many organs including the gastrointestinal tract, pancreas, adipose tissue and the brain are still under development in infancy and toddlerhood. Body size doubles and body weight increases five-fold in this period of time. Two grams of brain are added every day[1]. In this critical phase of childhood, the foundation is laid for a healthy life. The risk of becoming overweight in childhood starts in the first year of life. Subsequent rapid increases in Body Mass Index (BMI) further aggravate the risk of becoming obese[2,3].

It is thought that the maturation of organs is adapted to the nutritional environment in this critical period of development. An excess of energy, unbalances in macronutrient quality or nutritional deficiencies are inappropriate nutritional signals, which may lead to e.g. metabolic disturbances or the development of obesity[4-7]. Early life is also a crucial phase for the development of healthy eating habits. There is a window of opportunity to form food preferences, and by repeated exposure and offering variety, children learn to accept many different tastes[8-10] and these preferences subsequently track into childhood and beyond[11-14]. In this review, we summarise the possible negative effects of

excess sugar and sweetness in products for infants and young children. At the end of the paper we share the commitment and approach of Danone Nutricia Early Life Nutrition in improving its portfolio.

Increased risk of childhood obesity

There has been a dramatic increase in the prevalence of childhood overweight and obesity in the last three decades in developed countries, and the problem is emerging in low-income countries[15]. Excess weight is associated with a higher prevalence of cardiovascular and metabolic diseases, including Type 2 diabetes, dyslipidemia, hypertension, atherosclerosis and non-alcoholic fatty liver disease later in life[16]. The number of studies investigating the relationship between total sugar intake in children and obesity risk is small, partly because of the small number of countries that have good sugar intake data. Studies conducted in populations of children demonstrate positive associations between intake of sugar and BMI[17-19].

In recent years there has been growing interest in the role of fructose in obesity and metabolic disease. After its introduction into foods, there has been a concomitant increase in obesity over the same period. To date however, no compelling evidence for a specific effect of fructose on obesity or metabolic disease has been shown[20,21].

A more strongly established relationship is between the intake of sugar coming from sweetened beverages and body weight development, possibly because liquid sugars do not give the right sense of satiety. Several observational studies indicate that a greater consumption of sugar-sweetened beverages, including sugared fruit juices and drinks and carbonated beverages, is associated with weight gain and obesity[22,23]. In an intervention study by de Ruyter *et al*[24], sugar-containing beverages were replaced by non-caloric beverages in normal weight children. A reduction of weight gain and fat accumulation was observed. There seems to be sufficient evidence for public health strategies which discourage overconsumption of sugary drinks[25].

Negative impact on the development of healthy eating habits

Human infants are born with an innate preference for sweet, shown by typical facial expressions indicating relaxation when they are exposed to sweetness[26,27]. Sweet foods are often energy-rich, which may explain the preference for sweetness, as energy is needed for optimal growth and development[28,29]. Most infants and toddlers prefer a sweet solution over water[30] and breast milk also has a sweet taste[31]. During the first year of life, inter-individual differences in sweet taste acceptance become bigger[11,12]. Experience with sweet foods and beverages may modulate a child's sweetness preference and their consumption pattern. Early exposure to sugar increases the preference and consumption of sugared and sweetened products later in childhood[11-14].

Interference with the intake of other nutrients

Another suggested adverse consequence of excess sugars is its effect on the intake of other nutrients. Most studies do not find an association of sugar intake with total energy intake. As fruit juices may replace milk drinks when infants get older, a shift in micronutrient intake may occur[32]. Intakes of specific micronutrients such as calcium, zinc, thiamin, riboflavin, niacin and folate have shown to be reduced with increasing sugar intakes, but generally stay well above recommended intakes for children[17,33].

Figure 1: The results of a sugar reduction program launched by ELN in 2010

Increased risk of dental caries

Dental caries is a bacterial infection that causes demineralisation and destruction of the hard tissue of the teeth. It is a result of the production of acids by bacterial fermentation of foods that are present in the mouth. The form of caries seen in young children is called Early Childhood Caries,

which can affect both the front teeth as well as molars. Sucrose is highly cariogenic and a study by Karjaleinen *et al* shows that sucrose intakes at three years of age is higher in those who developed caries by the age of six[34]. Similar results were shown in the British National Diet and Nutrition Survey (NDNS) of pre-school children aged 1.5 to 4.5 years in which the consumption of non-milk extrinsic sugars was related to caries incidence[35]. The relationship between sugars and dental caries is complex as oral hygiene is a confounding factor. Reducing frequency of consumption of sugar containing foods and improving oral hygiene may have a large impact on incidence of dental caries.

Reducing sugar and sweetness in products for infants and young children

Danone Nutricia Early Life Nutrition (ELN) has a strong commitment to limit the amount of sugar in products intended for infants and young children. Specific minimum and optimum nutritional standards have been developed per category as reference values for product development. All categories are included, such as milk-based formulae, growing-up milks, cereal-based foods, savoury dishes, fruit-drinks and dairy based dishes. Minimum standards are mandatory for all products in the ELN product portfolio. They are there to help us continuously push for improvement, while at the same time taking into consideration the variations in eating habits and cultural practices in our markets around the world. Optimum standards represent our final ambition and will drive the nutritional quality of our products in the long run. Optimum standards are based on the latest science and are set to deliver the most significant benefits to infants and toddlers. The optimum standard for sugar content for all these categories is 'no sugar added'. ELN aims to meet optimum standards in as many products as possible. A sugar reduction program was launched in 2010, with the aim to reduce 20 per cent of sugar in products by 2015. **Figure 1** (page 34) shows that the actual reduction was almost 14 per cent by 2012 and the first estimates for 2013 are 15 per cent reduction.

Several challenges can be identified when reducing sugar in products intended for infants and young children. Although it is relatively easy to get young infants to adapt to a non-sweet taste[36], the mothers' taste is often the reference. She will try the food and may reject products that are not sweet enough. Cultural determinants in a preference for sweet foods may be strong. The risk is to lose consumers to non baby-specific foods or competitor products with high sweetness. In addition, parents are not always interested or aware of the long-term health consequences of excess sugar intake.

Successful launches of reformulated products within ELN were often done without any specific communication to parents or health care professionals. A stepwise reduction of sugar was shown to avoid losing consumers after launch.

In summary, reduction of sugar and sweetness is relevant and realistic in products intended for infants and young children.

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Enrobing in the confectionery industry

Enrobing is a process that involves covering a confection or snack with chocolate or chocolate coatings. Traditionally, this process was slow and involved manually dipping the pieces into melted chocolate by hand. As demand for chocolate-coated sweets increased, it became impractical or impossible to employ enough people to dip sweets into melted chocolate to keep up with production demand. Enrobing can be carried out with chocolate or compound coatings (compound coating is a replacement product made from a combination of cocoa, vegetable fat, and sweeteners). An advantage of compound coatings is that they may set faster and no tempering (the process in which chocolate masses are thermally treated to produce a small fraction of homogeneously dispersed, highly stable fat crystals of the correct type and size) is needed[1]. Some typical examples of enrobed products are shown in Figure 1 (page 37). They include wafer bars, fondant centres, jellies, nuts, biscuits and ice cream.

Through covering the centre with chocolate or compound coatings, the shelf-life of the product may be extended. This is primarily applicable to centres that, if not covered, could be prone to moisture uptake/loss, oxidation, or microbial spoilage. Enrobing has some advantages over moulding (which is another method of getting a chocolate covered centre) such as greater production rates, lower capital costs. Enrobing often allow for greater production rates with lower capital costs than moulding[2].

Enrobing process

Enrobers are available in different sizes, suitable for large and small scale production and there is a wide variety of different designs to meet all requirements. Belt widths from 125 millimetres to 2600 millimetres are available. Although the basic elements of an enrober have largely remained unchanged over the years, the methods of operation and degree of precision possible have changed significantly. This has been accompanied by a modest increase in throughput. The biggest change in the manufacturing of chocolate enrobed sweets can be credited to the efficiency of the coolers. The basic layout of an enrober is shown in **Figure 2** (page 37).

Processing a real chocolate always requires a tempering unit. Whether fitted with a temperer internally or externally, enrobers have the same basic components (**Figure 2**, page 37). It is important that the centres entering the enrober are maintained at 21-24°C, and that the enrobing chocolate has the desired viscosity and rheological properties. Warmer centres may lead to possible bloom problems due to the

Figure 1: Some examples of enrobed confectionery

residual heat increasing the chocolate temperature of the enrobed sweets. Cold centres can lead to blooming and cracking of the coating shell due to expansion of the centre mass as it warms. Fat bloom develops in different ways. Automatic crystal conversion on the one hand caused by incorrect and/or insufficient tempering; on the other, it may be caused by fat migration from the filling where this fat penetrates the chocolate coat and causes the cocoa butter crystals to rise through the surface. Loss of temper can also be due to heat damage.

The centres are fed on to a feed band and transferred to a wire belt, which passes through the enrober. The coating medium is maintained at a constant temperature and in a controlled condition in an agitated tank; it is then pumped to a flow pan. The flow pan aids the process by creating a continuous curtain of coating and feeding a bottoming device. This

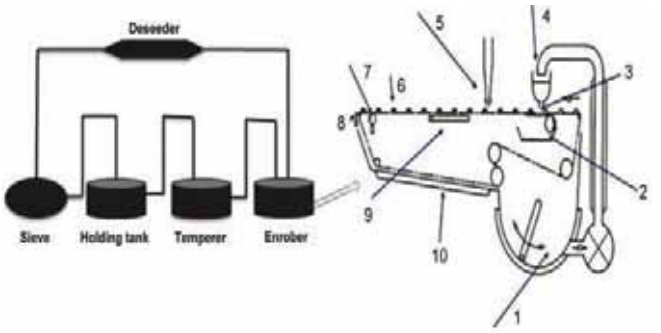


Figure 2: Basic layout of enrobing line and components of an enrober[2]
(1) Sump; (2) Bottomer; (3) Flow pan; (4) Riser tank; (5) Blower (6) Wire belt; (7) Licking roller; (8) De-tailing rod; (9) Shaker; and (10) Heated extension trough

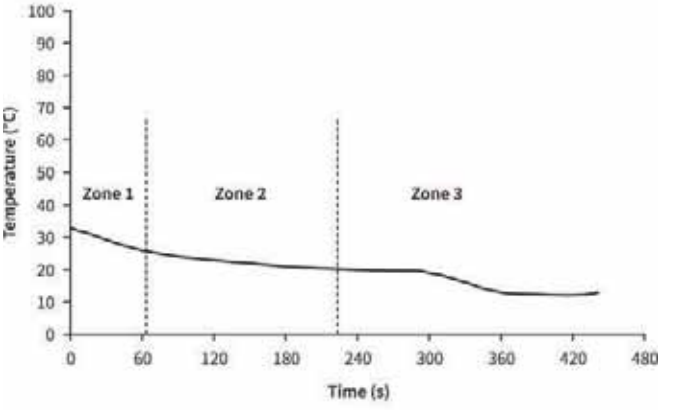
leads to the formation of a bed of coating, which floods the mesh band. The centres are passed through this curtain and bed and are covered on all surfaces. After the curtain, excess chocolate is forced off the product by an air blower and a licking roller is used to control the amount of mass left on underside of the sweet. There is normally a vibrator after the blower to remove excess chocolate and to improve the appearance of the sweet. Finally, there should be a detailing rod between and end of the wire belt and the start of the cooler belt. Following the curtain, using an air blower, the excess chocolate is removed and a licking roller controls the amount of mass left on the underside of the sweets[3].

The excess mass from the curtain falls through the wire mesh belt into a sump, and is recirculated. Part of the mass is diverted through a de-temperer and is then re-tempered; blending of the freshly tempered and recirculated streams controls the overall level of chocolate in the enrober. Vibrating the centres removes ripples left by the fan, smoothens the coating and removes any surplus chocolate returning it to the tank. The centres are discharged from the enrober on to a cooling conveyor passing over a de-tailer which is a rapidly spinning rod across the end of the wire band. This results in the removal of the tail that forms as the centre leaves the wire band.

Cooling

After enrobing, the product enters a cooling tunnel to allow the coating to harden. To avoid blooming problems, temperature changes in the tunnel should be gradual, and the relative humidity properly controlled. If the dew point is lower than room temperature, moisture could condense on the product and cause sugar bloom during storage[4]. The chocolate coating and the filling are cooled down to approximately 18°C to ensure trouble-free packaging. A good cooling tunnel should be divided into three zones (Figure 3).

Convection chocolate cooling is a time-dependent but not energy-dependent process. A higher temperature and longer cooling time are more favourable than a lower temperature and shorter cooling time. The recommended cooling times for pure dark chocolate, milk chocolate and milk chocolate with CBE portions are approximately six, eight and 12 minutes respectively. Milk chocolate requires a longer cooling time than dark chocolate due to the higher milk fat content and consequent lower solidification temperatures. Compound coatings may



Zone	Type of Heat transfer
1	In the tunnel inlet, only sensitive heat is removed from the centre and/or the chocolate coating. Recommended heat transfer is by air circulation around the product (convection).
2	Approximately 3 minutes, the crystal growth is started. Up to this point gentle cooling required. Latent heat is removed from the chocolate. The recommended heat transfer is by exchange without air circulation (Radiation).
3	Both sensitive and latent heat is removed in the tunnel outlet.

Figure 3: Temperature profile in the cooling tunnel[5]

require different cooling profiles i.e. adjusted to suit the setting properties of the vegetable fat used. After cooling, a product which is shiny and resistant to handling should be available for packaging.

Product attributes

Physico-chemical properties of enrobed products are important for two reasons. Firstly, if the crystallisation or flow properties are incorrect this results in a poor quality product being made, which may have to be sold cheaply as a misshapen product or perhaps has to be reworked. Secondly, sensory attributes are critical to the consumer's appreciation.

An enrobed product is unlikely to be purchased of if it does not look glossy, or worse still, if the fat has bloomed. One important visual characteristic of enrobed products is gloss. This is determined by the reflected light. If the surface is flat with a lot of small crystals, which happens with correct tempering and cooling, the product appears shiny[6]. Cocoa butter can solidify in different forms of crystals, where only the high melting forms β_v and β_{vi} are stable. A tempering machine can only effect formation of the crystal form β_v. This crystal form provides good gloss, long shelf life and good mouldability. The β_{vi} form is a super stable crystal, which forms only after a longer period of storage[6].

Chocolate or compound flow properties are decisive factors in terms of processing possibilities. The flow properties of a coating are very complicated because the viscosity is not a single value but is dependent on the speed of flow (technically as non-Newtonian fluid). Yield value (YV) is the shear stress required to initiate the flow of a coating. YV is recorded in Pascal (or dynes/cm^2). Plastic viscosity is then the force needed to maintain this flow once it is moving, which is recorded in Pascal seconds[7].

Enrobing of high-quality confectionery items nearly always requires a low-viscosity coating with some reasonable yield value present. The viscosity depends on the level of fat, emulsifier content, temper profile, particle size distribution and temperature. Low viscosity is required for quality lines, giving precise control of coverage to the enrober operators[8]. Without some yield value present, the chocolate would continue to flow down the side of an enrobed piece before setting in the tunnel, resulting in 'skirts' or 'feet' on the bottom edge of pieces, and decorative markings would be lost (**Figure 4**).

Figure 4: Effect of Yield Value on enrobing

Generally, bubbles are not a major problem on enrobed items as the coating is fluid enough for them to be displaced or burst by the blower. However, with thicker masses or some more difficult products, bubbles can be problem and action may be needed to minimise them. Each feed pipe to the curtain trough should have its outlet under the chocolate surface to avoid incorporating air.

Conclusions

Enrobing creates opportunities for manufacturers to create varied, decorative (for example through the use of irregularly shaped inclusions) confections to the ever-demanding consumer. Enrobing also presents some advantages over moulding, by often allowing for greater production rates with lower capital costs. With compound coatings, one can increase speed even more due to quicker setting times.

The basic requirements to enrobe confectionery products have remained unchanged for decades, however the methods used to control their operation and the degree of precision possible have changed significantly, accompanied by a modest increase in throughput. Products such as wafers, soft centres or cookies can be coated fully or partially with chocolate or compound coatings. Special attention is paid to ensure reproducible and accurate product weights and a uniform bubble-free appearance. Perhaps the biggest change in the manufacture of chocolate enrobed confections has been in the efficiency of the coolers. After cooling, a shiny and uniformly coated product should be available for packaging.

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About the Authors

Dr. Ramana Sundara is a Fellow and Chartered Scientist of the Institute of Food Science and Technology (UK). Currently, he is the Manager of External Research Collaborations at Nestlé Product Technology Centre, York, UK. He has published over 34 scientific papers / presentations and five patents, with an emphasis on fruits, vegetables, chocolate, dairy chemistry and processing technology.

Ángel Máñez is a Principal Product Technologist at Nestlé Product Technology Centre for confectionery products in York, UK. Ángel joined Nestlé as a post graduate student and first worked on research projects on the chemistry of cocoa fermentation. Subsequent to that he gained experience during two to three years in the area of cocoa processing. For the last 15 years with Nestlé, his main area of expertise has been closely related to chocolate manufacture and usage and in particular chocolate recipe formulation. As part of his present role he provides technical assistance to factories, participates in projects to develop new products and processes and also participates in the commissioning of new lines in factories.

Dr. Josélio Vieira is a Principal Research Scientist at the Nestlé Product Technology Centre for confectionery products in York, UK. Josélio is a Chemist by training and holds a PhD degree in Physical Chemistry from the University of Oxford. After graduating, he worked for 11 years at Dow AgroSciences in the development of crop protection formulations in the Formulation Science & Technology group in Brazil and the UK. He then joined Nestlé at the Product Technology Centre in Beauvais, France, dedicated to ice cream product technology development. After five years in France, Josélio was relocated to York where he now works in the Chocolate Department. His interests include colloidal and formulation sciences. Josélio has co-authored and published a number of research papers and patents on chocolate and ice cream technologies.

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Ashlee Breitner Business Unit Manager, NSF International

ISO 21469: Why this International Standard is on the rise

The uptake of global food safety standards in recent years has prompted sectors throughout the food safety supply chain to implement more rigorous product safety measures, and the lubricants industry is no exception. In 2006, the need for an internationally recognised set of requirements was made apparent to the International Organisation for Standardisation (ISO). This need was to establish a new, voluntary ISO standard for lubricants used in the manufacturing and processing of food and similar products. The document was titled 'ISO 21469: 2006(E) – *Safety of machinery – Lubricants with Incidental Product Contact*', by the Technical Committee ISO/TC 199, Safety of Machinery, and it specifies the hygiene requirements for the formulation, manufacture and use of lubricants which may come into contact with food products during processing.

The scope of this international standard goes beyond lubricants used in food applications to also cover lubricants used for processing high risk products including cosmetics, pharmaceuticals and animal feed. The intention behind the broadened scope of ISO 21469 is to provide additional risk mitigation solutions for other product categories where hygiene standards in manufacturing are of particular concern.

You may be asking why an article about a standard published in 2006 is so relevant now. In the past two to three years, certification to

ISO 21469 has gained traction and now making an impact on the food grade lubricant industry and food safety proponents are taking notice. As more lubricant companies and production locations achieve ISO 21469 certification, and increasing audience of stakeholders – end users, regulators, and lubricant manufacturers alike – are feeling the benefits.

"ISO 21469 goes beyond the requirements of H1 and covers the whole lifecycle of the lubricant. The lubricant manufacturer is required to analyse the hygiene aspects that arise from handling a lubrication product, and to advise the user accordingly. So a food and beverage producer can be assured that every effort has been made to take their safe usage and hygiene requirements into account; all this whilst delivering long-term lubricant performance and equipment protection," stated Jesus Diaz, Market Manager at Klüber Lubrication München Se & Co. Kg.

certifications to ISO 21469 are strategic tools that assist companies in tackling the most demanding challenges facing businesses today

What does international recognition mean?

The development of this standard like many others was intended to ensure uniformity of product safety and quality with a particular industry. The ISO 21469 standard appeals to lubricant manufacturers seeking compliance to a single, internationally accepted standard that is comprehensive enough to address lubricants used across multiple industries and product sectors. International acceptance of this standard helps companies to gain access to new markets and communicate the safety and compliance of their products to end users, regardless of geography.

While international standards facilitate harmonisation of product requirements, they also serve to facilitate more effective industry coordination and eliminate barriers to international commerce. For countries where the food grade lubricants industry is less developed, having an international standard levels the playing field for companies seeking to export their products into regions with higher lubricant demand. For example, the Emirates Standardisation & Meteorology Authority (ESMA), the official federal body in the United Arab Emirates, announced adoption of ISO 21469 as the mandatory requirement for incidental contact lubricants. This adoption in itself exemplifies significant progress in minimising lubricant trade barriers for the Middle Eastern market.

In addition to facilitating trade, certifications to ISO 21469 are strategic tools that assist companies in tackling the most demanding challenges facing businesses today. Efficiencies, cost savings, streamlined processes, and risk mitigation are key issues as companies work to operate effective businesses and increase productivity. Achieving certification to an internationally accepted standard can

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translate to cost savings from optimised operations, enhanced customer satisfaction based on improved product quality, and increased market share resulting from a competitive marketing advantage. Further, certified companies may also reap environmental benefits by reducing the negative impacts from disposing of contaminated or adulterated products.

As one of the leading producers of food grade lubricating oil and greases, Mrs. Zhaoying Yu-Zhu, Sales – Asia Area & Food Grade Group Product Manager of Axel Christiernsson has stated that they "are very proud that our manufacturing facility dedicated to food grade products, has been certified NSF ISO 21469 since 2009. 'NSF H1 certified' is a good way to guarantee our products meet the food grade lubricant requirements. In addition, certification by NSF to ISO 21469 provides us a stronger safety insurance regarding manufacturing process towards our customers. With ISO 21469 certification, it allows us to differentiate compared to other non-certificated manufacturers. This also gives us a competitive advantage to expand our distribution internationally."

How is ISO 21469 raising the bar?

Companies seeking to demonstrate their commitment to quality by applying for ISO 21469 certification are thoroughly evaluated to ensure that their products meet particular hygiene requirements for the formulation, manufacture, and use and handling processes of lubricants that may have incidental product contact. ISO 21469 certification requires lubricant manufacturers to develop a hygiene strategy and to consider chemical, physical and biological hazards in the context of the lubricant end use.

Figure 1: Once all assessment elements have been completed satisfactorily, products can then be marked as ISO 21469 certified

ISO 21469 certification covers four key assessment elements for achieving and maintaining annual certification. These include: 1) an initial formulary and label review of the ingredients in the lubricants; 2) annual testing of certified product; 3) a completed risk assessment for all products certified; and 4) annual production facility audit.

Formulary and label review

The company or its suppliers shall submit complete formulation information for all components of a product submitted for evaluation

and certification. Ingredients used in certified lubricants with incidental product contact shall meet specific regulatory and safety requirements, for the appropriate end use of the product.

The label review for certification ensures that language specified on the label of a certified product clearly and correctly identifies the following: product shelf-life, storage conditions, product size / volume, suitability of the product for use as an incidental food contact lubricant, and any product limitations and / or restrictions. The label review also verifies that products requiring registration under the US Environmental Protection Agency's (EPA) Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), are appropriately registered.

Food adulteration due to contamination by traditional lubricants can result in product recalls and be costly, both to the bottom line and a company's reputation

Testing

Product samples are to be analysed by Fourier Transform Infrared Spectroscopy (FTIR). FTIR analysis provides a highly specific measure of material identification by comparison of a test sample spectrum with a reference spectrum. This annual testing serves as a quality control check in the ISO 21469 certification to again verify the formulations of the certified product are consistent in their qualitative composition.

Risk assessment

Certification to ISO 21469 requires that the lubricant manufacturer complete a risk assessment addressing the potential sources of contamination during production of the lubricant. The risk assessment is designed to ensure that all potential risk factors in the possible contamination of a certified lubricant have been defined, measured and quality controls have been set in place to ensure that those risks are mitigated on an ongoing basis.

Audit

In order to gain and then maintain certification, an annual production facility audit must be conducted and corrective actions resolved. Audits are a critical piece to aide in ensuring that the products originally certified are those that are being produced each and every time production is run. Audits include actual production facility walk-through to inspect maintenance of equipment in accordance with Good Manufacturing Practices, verification of risk management procedures, review of supplier qualification records, verification of raw materials, review of labeling of certified product and any additional control measures necessary to guarantee quality product. Additionally, the audit scope may be influenced by corrective or preventative actions or other necessary measures for minimising hazards identified in the risk assessment.

At the point in which all of the elements have been completed satisfactorily, products can then be marked as ISO 21469 certified (see **Figure 1**, page 44).

As stated by Rocol Lubricants: "The positive effects of ISO 21469 are felt by many stakeholders: Lubricant manufacturers seek ISO 21469 accreditation as the highest accolade that can prove their suitability for food grade applications. Auditors are reassured by the presence of accreditation as it allows full traceability of the lubricant manufacturing process. Food manufacturers prevent contamination of their products and reduce waste whilst supplying safe products to major supermarkets and other distributors. Consumers are protected against the possibility of eating a product which is tainted with hazardous material. Once ISO 21469 certification is achieved it proves that a lubricant is manufactured in a hygienic environment, using both best practices and the safest ingredients." The impact that ISO 21469 certified products have on all stakeholders of the lubricant industry is significant. Effectively managing risks in their supply chain is priority number one for today's food and beverage companies.

ISO 21469 is a voluntary standard but the food industry has already recognised the value and benefits of it as they are proactively adopting ISO 21469 into their lubricant purchasing specifications. The value of ISO 21469 certification for end-users is that it provides added assurance that the lubricant formula meets food safety requirements, label information is accurate and traceable and lubricant manufacturing and packaging conditions are hygienic.

Food adulteration due to contamination by traditional lubricants can result in product recalls and be costly, both to the bottom line and a company's reputation. The benchmarks the lubricants industry is striving towards – increased efficiency, cost reduction, streamlined processes, and risk mitigation – can be synonymous with making a safer lubricant product. For companies focused on protecting and improving the integrity of the food supply chain, ISO 21469 is the standard that helps brings both worlds together.

To access the most current list of ISO 21469 certified products, please visit: www.nsf.org

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Ensuring productivity: how food grade lubrication certification provides best practice

Effective lubrication of food processing equipment is increasingly important, with manufacturing operations being expected to run continuously and equipment having to endure conditions such as extreme temperature swings and contamination-prone environments. Frequent water washouts can contaminate and wash away lubricant, and all of these factors can affect the productivity of machinery; making it more important to choose the right food grade lubricant. There are also rigorous certifications that need to be achieved to ensure equipment lubricants are manufactured in a hygienic environment, using both best practices and the safest ingredients.

Eduard Stempfel, Global Product Manager and Application Specialist at FUCHS LUBRITECH, explains that although the same production facilities can be used for food grade lubricants as well as for standard products, more stringent rules are applied in order to achieve the highest purity levels and avoid the possibility of cross-contamination. “Production facilities must be certified to ISO 9001/14001 as a minimum for the manufacture of lubricants for the food/feed and beverage processing industry. Since 2006, the ISO 21469 standard specifies

the hygienic requirements for lubricants with incidental product contact," he explains.

There is, however, a significant difference between product registration only (e.g. H1) and ISO 21469 certification. The ISO 21469 consists of a full risk assessment for the entire lubricant manufacturing plant and a physical yearly audit including formulation review, process review and sample taking and testing. ISO 21469 currently represents the highest standard for food safety, specifically dedicated to manufacturing and handling of food grade lubricants. "Including FUCHS, there are only seven companies (11 legal entities) with 11 different lubricant manufacturing plants currently certified against this standard by NSF International," reveals Stempfel.

According to Stempfel, there have been an increasing number of lubricant suppliers who have incorrectly claimed their products are ISO 21469 compliant over the years just because compliance does not necessarily mean certified. NSF International's ISO 21469 certificaiton programme is accredited by the American National Standards Institute (ANSI) and provides independent, third party assessment of a lubricant's compliance with the hygiene requirements established by the standard. NSF International is the only organisation currently carrying out full physical audits following an accredited scheme, which includes full risk assessment, sample testing and formulation and paperwork review. "Food and beverage manufacturers should investigate the information they have been told by their lubricant supplier," suggests Stempfel. "It's just a case of visiting the NSF ISO 21469 certification website which is a transparent and verifiable approach that ensures there are no questions as to whether a company or manufacturing plant is ISO 21469 certified.

"NSF ISO 21469 certification combined with NSF H1 registration assures the food manufacturer that the product is the safest food grade lubricant. This, in turn, means that when the food manufacturing plant is audited for food safety according to IFS ISO 22000 or other standards, the auditors will recognise that the lubricants in use are of the highest safety standards and can be eliminated as potential chemical or physical hazard sources in the plant's HACCP audit. NSF ISO 21469 certification is a very important contribution of a lubricant manufacturer to the Global Food Safety Initiative (GFSI). FUCHS is committed to the GFSI and the FUCHS group of companies currently offer more than 160 NSF ISO 21469 products from three different ISO 21469 certified manufacturing plants."

UK food grade lubricants specialist ROCOL® is working more closely than ever with food and beverage processors to help eliminate plant and machinery safety risks

UK food grade lubricants specialist ROCOL® is working more closely than ever with food and beverage processors to help eliminate plant and machinery safety risks. "A year on from the horsemeat scandal, and in an era of even greater food chain traceability, we are working to eliminate safety risks and restore consumer confidence," says Joanne Ferguson, Marketing Manager at ROCOL®. "Since that scandal, total supply chain integrity has become vital to give retailers and consumers credible assurances about product safety – and for processors this includes being careful about lubrication choices. Making the right choice means insisting on the use of independently-certified NSF H1 registered lubricants only."

NSF H1 registration demonstrates that a product has been reviewed by NSF to ensure it complies with the latest safety standards. Once checked and certified, the lubricant is deemed safe for use in factories producing food, drink, cosmetics and animal feeds, and is guaranteed to be manufactured only of 21 CFR compliant ingredients. According to Ferguson, for more comprehensive assurance, processors should also look for lubricant manufacturers who are ISO 21469:2006 certified: "Like NSF H1, this certification is globally recognised and ticks an important box for EFSIS/BRC auditors as it provides highly credible, independent assurance that products are formulated, manufactured and supplied hygienically and safety. So important is ISO 21469:2006, that in 2010 ROCOL® took the decision to invest in becoming the first UK lubricants manufacturer to achieve certification; enabling processors to take advantage of this additional layer of safety assurance."

ROCOL® has developed smart solutions for ever more specific applications, which keep machinery at peak performance without compromising on safety. And thanks to the company's in-house research and development team, its FOODLUBE® range of food grade lubricants continues to grow. Ferguson explains: "Typifying ROCOL®'s continuous innovation over the past 12 months is the company's new FOODLUBE® Auto SF – a food grade NSF H1 registered semi-fluid grease which delivers efficient and effective lubrication in even the smallest bore pipework, and was specifically developed to overcome the potentially costly issue of pipe blockages and splitting in automated lubrication systems with clear processing environments.

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Lubricant manufacturer Lubriplate Lubricants has a complete line of high-performance NSF H1 registered food machinery grade lubricants that are designed to provide total food processing and bottling plant lubrication capability. "Manufactured in compliance with strict ISO 21469 and ISO 9001 registered quality assurance standards, these lubricants are formulated from the highest quality base stocks combined with state-of-the-art anti-wear additives. They deliver a number of significant, cost effective advantages, which include extended lube and fluid change intervals, multiple application capability, lubricant inventory consolidation and improved performance," explains Jim Girard, Vice President and Chief Marketing Officer at Lubriplate Lubricants Company.

"All Lubriplate H1 lubricants are manufactured with ingredients that comply with FDA regulation 21 CFR 178.3570 for lubricants with incidental food contact. They meet NSF H1 safety standards and are authorised for use in federally inspected meat and poultry plants. Clean, safe and non-toxic, their use can eliminate lubricants and lubrication as potential chemical hazards in HACCP programmes."

Lubriplate NSF H1 lubricants include: Synthetic fluid lubricants and greases; USP white mineral oil-based oils and greases; oven chain lubricants; refrigeration compressor fluids; cleaning and flushing fluid; and H1 aerosol spray lubricants. Many Lubriplate NSF/H1 registered greases are formulated with a USA Environmental Protection Agency-registered anti-microbial additive. "Our products also come with the exclusive, complimentary, Lubriplate ESP Extra Services Package, which includes a plant lubrication survey by a factory direct representative, lubrication scheduling software, machinery lubrication tags and follow-up lubricant analysis," says Girard. "The lubricants are available through master distributor FINKE Mineralolwerk GmbH."

Finding a lubricant that meets stringent food safety standards, provides lubrication even

under tough conditions helps to reduce downtime and saves time and money, is a challenging task for even the most experienced plant manager. “Without regular maintenance and proper lubrication, food contamination of the lubrication system can also cause harm to a plant’s vital equipment,” explains Michael Colquhoun, Category Portfolio Manager – Food Grade Lubricants at Petro-Canada Lubricants.

Confectionery processing is one area in which cost savings and increased equipment performance can be achieved. Chocolate in particular is an exceptionally fine and abrasive product which makes quality lubrication essential to manufacturing processes. For example, Cadbury Limited’s production facility, located in North Wales, faces unique maintenance challenges due to the nature of its product. “The Cadbury site produces over 7,000 tonnes of finished product (drinking chocolate) and around 4,000 tonnes of bulk material (cocoa liquor, cocoa butter, cocoa powder) per year, for use at other Cadbury manufacturing sites,” says Colquhoun. To help manage this, Cadbury enlisted the help of Paul Needham of AV Technology at Cadbury Limited, to monitor and handle their equipment maintenance. In providing the Reliability Engineering consultation for Cadbury, AT Technology switched the lubricant used in Cadbury’s Duyvis Cocoa Presses to Petro-Canada’s PURITY™ FG AW 46 Hydraulic Fluid.

Needham explains: “Switching to Petro-Canada Lubricants’ PURITY™ FG has reduced costs, increased our up-time and improved the performance of our equipment. We have over 10 years of experience with Petro-Canada and are pleased with the performance that their fluid has provided.”

The hydraulic system in the cocoa press has a capacity of 1,015 litres and works at a system pressure of 510 bar. With this pressure system along with the product’s fine, abrasive powders, a strong lubricant is needed to protect the equipment. “Using premium products like Petro-Canada Lubricants’ PURITY™ FG food grade line, in combination with proper maintenance procedures, can help extend service life, lower fluid consumption and reduce operating costs,” explains Colquhoun.

With more than 30 years of experience in manufacturing, formulating and blending Group II and III base oils, Petro-Canada Lubricants delivers a diverse line of high quality, innovative lubricants to meet an ever-increasing range of international specifications. Colquhoun concludes: “PURITY™ FG lubricants are NSF registered, acceptable for use in Canadian food processing facilities and have Kosher, Pareve and Halal certifications. PURITY™ FG lubricants are a perfect fit for Hazard Analysis and Critical Control Points (HACCP) and good manufacturing practice.”

UmBio

APPLICATION NOTE/FOOD

Do you know what's on your dinner plate?

NIR HYPERSPECTRAL IMAGING:

Beef or pork? Real or fake? Near real-time characterisation of meats

Consumers have become distrustful of meat and meat containing food products because of the ever increasing cases of meat adulteration during recent years. A few examples include the dyed and chemically treated pork, and horsemeat sold and misrepresented as beef. Detection of species adulteration in meat products is important for consumer protection. Proper food labelling is essential in order to prevent misleading consumers. Importantly, as there are religious limitations of certain food products such as pork meat and some people are allergic to certain meat proteins, a method that enables rapid discriminatory analysis of meats is urgently needed.

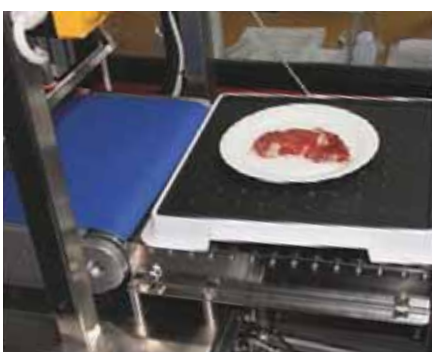

Figure 1: Analysis set-up: raw meat on a paper plate was removed from cold storage for analysis with the UmBio Inspector. The meat was returned to cold storage seconds later.

Species identification in meat has proved to be difficult, costly and time consuming. A range of variables influences raw meat, for example breed, age, feeding, storing time and conditions. Fresh, raw meats also require fast and careful handling and thus its analysis must be rapid. Here, we present a method for the detection of raw beef and pork meat using Hyperspectral Image Analysis.

Results and discussion

All meat observations were converted into objects and subjected to a PLS-DA analysis. The result reveals three distinct clusters (Figure 2). Pork and beef were clearly separated along the x-axes (t1) and beef tenderloin and beef rib eye were separated along the y-axes (t2) indicating a trend for different fat content along t2.

Interestingly, the pork tenderloin and the marinated pork tenderloin were grouped together (Figure 2). This indicates that the marinade and the difference in colour do not influence the detection of pork meat in dimension t1 and t2.

The hyperspectral image also indicates three groups (Figure 3). This is shown as different colour schemes. Both types of pork tenderloins have an orange colour compared with the beef steaks that are more turquoise / yellow. The beef rib eye has a greener colour shade than the beef tenderloins. Fat is coloured in blue (Figure 3). Hyperspectral Imaging makes detection, discrimination, classifica¬tion of meat and meat products easier. This can be applied in routine quality control for the food industry.

Experimental set-up

Vacuum packaged raw meats (beef tenderloin, beef rib eye, pork tenderloin and marinated pork tenderloin) were sliced and placed on disposable paper plates and analysed with the UmBio Inspector (Figure 1). The hyperspectral image cubes were generated in diffuse reflectance with a line scanning (push-broom) short wave infrared (SWIR) camera in the spectral range of 1000 – 2500 nm. The data was converted automatically to pseudo-absorbance and processed with the hyperspectral image analysis software Evince (UmBio AB, Umeå, Sweden).

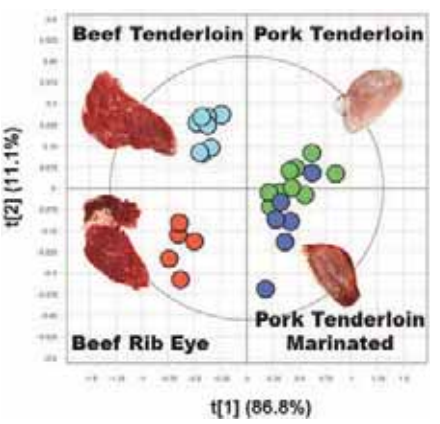

Figure 2: PLS-DA score plot revealing three distinct clusters. Beef and pork are separated along t1.

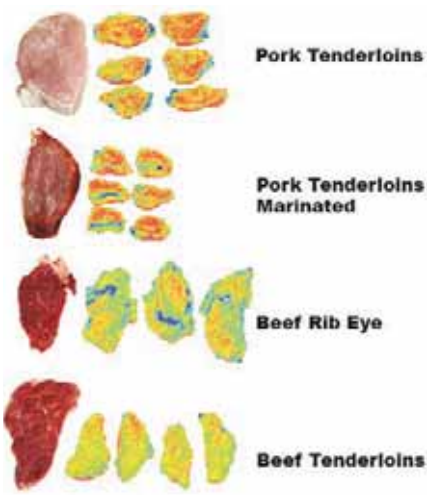

Figure 3: A Hyperspectral Image generated from the analysis. Variations between the different types of meat are depicted as different colors which enable fast and easy detection.

Conclusion

- The UmBio approach offers rapid and non-destructive analysis on all types of meat
- This technique permits analysis of multiple individual meat products simultaneously
- Characterisation of meats can be performed in real time
- Deviating products are detected in seconds
- Fresh meats may be returned to cold storage within seconds
- No sample pre-preparation, no solvents, and no wet-chemistry laboratory were needed in this analysis and no hazardous waste was generated in this study.

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Food fraud and NIRS

Nowadays foods and ingredients are sourced from many different parts of the globe. Since the 1960s, global food transport has been increasing at an exponential rate, faster than food production itself. For certain countries, this network ensures access to any food item regardless of season or origin. The number of countries relying on international food trade has increased and traded food changed from raw materials towards processed and branded products. As a consequence, trade fluxes between countries to form a complex, extensive, intersecting network[1]. Optimised for rapid, low-cost production from all sources, it has consequently resulted in fragile networks that are vulnerable to food fraud that reaches every table in the world.

Food fraud may relate to compositional issues, e.g. certain constituents have been removed, added or substituted. Examples are product-foreign proteins or nitrogen-replacers in milk powders or hazelnut oil substituting olive oil, water addition to meat, husks to coffee, etc. Another category in food fraud concerns the product's history, i.e. geographical origin, production practice (organic, animal welfare considering, sustainable, halal, etc.), and processing.

Food fraud risk: prevention and detection

Although negligence of rules and agreements plays a role, premeditation is the main issue in food fraud. Food fraud differs from most safety issues in that fraudsters intentionally aim at deceiving targets by food adulteration or counterfeiting for their own economic gain. Food fraud is a crime of calculation, not of passion. Although the primary intention of the fraudster is not to harm people, it may still result in serious food safety issues. Examples include the melamine case in China in which several infants died, dioxin problems in oils of unknown provenance, and anti-freeze in wine.

Rather than looking at specific incidents, a system analysis approach would be the preferred approach to prevent fraud in the future. For instance, by studying why putting melamine in products was perceived as a good fraud opportunity and how it could have been prevented. Generally, three things must be present for a fraudster to set to work: the opportunity to commit fraud, the incentive to commit fraud and the fraudster's ability to rationalise their own actions[2].

Although risk assessments exist for food safety and defence issues, fraud temptation has never been taken into account. A science-based assessment of fraud risks requires an interdisciplinary, inter-sectoral

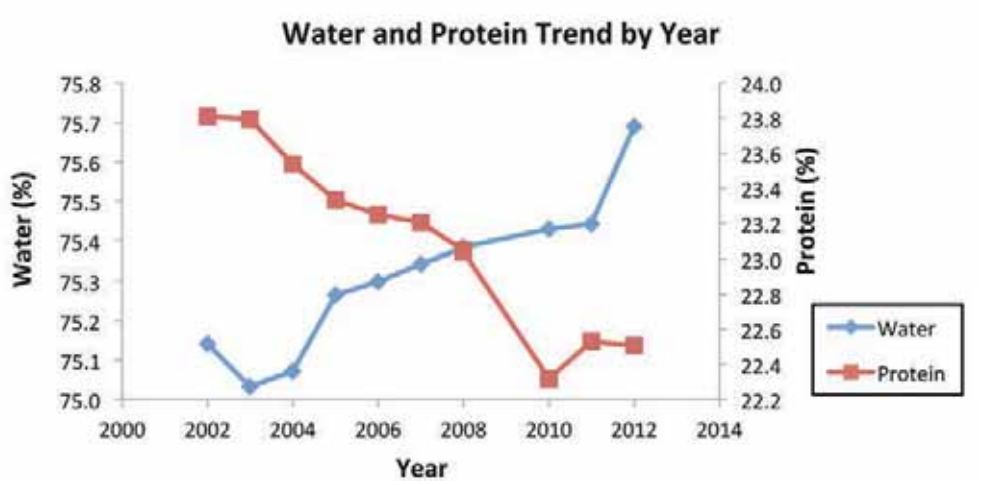

Figure 1: Change in water/protein contents in imported poultry meat in the Netherlands in the last decade measured by classical wet chemistry analysis

approach, i.e. an integrated analysis of technically feasible fraud openings, complexity of supply network structures, fraudster behaviour, safety management systems, as well as analytical detection methods. Since no one – producers, traders, importers, retailers and consumers – likes to be swindled, fraud prevention and detection is an important issue. Nowadays, authenticity of ingredients or products is mainly warranted by certification processes. Analytical tests which can help to confirm the authenticity of ingredients / products compose a very useful complementary approach to paper trailing.

Rather than looking at specific incidents, a system analysis approach would be the preferred approach to prevent fraud in the future

Analytical methods

Traditional strategies for food authentication rely on the determination of a marker compound or compounds and the comparison of the obtained values with those established for genuine material. Examples include macro-component contents such as protein, fat, moisture and ash. The presence of an undesired adulterant in the situation of compositional fraud can be uncovered by checking for its presence in the food material, e.g. melamine in milk powders. However, an adulteration can be detected only if the adulterant is known beforehand and explicitly searched for by the analyst based on this conventional target analyses. Although with multi-methods several potential adulterants could be detected, it is not practically feasible and financially impossible to analyse foods for all potential adulterants. Furthermore, some aspects of food authenticity such as the geographical origin, the farming management systems (organic, free range, etc.), or the application of some specific processes cannot be dealt with those traditional approaches. For instance, no single markers exist for the unequivocal authentication of organic eggs or sustainable palm oil.

For these issues, a more holistic approach is needed based on the measurement and the evaluation of a pattern of compositional properties of the product at once, i.e. a fingerprinting approach comparing analytical properties of groups of products while taking into account the natural variation. Moreover, in industrial and laboratory settings, there is always the need for screening methods that are able to reliably identify those products that are potentially non-compliant before more detailed and accurate analysis with confirmatory methods is performed, i.e. the traffic light approach. Fingerprints can be generated with a wide range of analytical techniques. They can be obtained from chromatograms, spectroscopic measurements, spectral measurements or any other specific signal of complete spectra. The approach requires chemometrics in order to elucidate relationships between samples and to detect characteristic patterns that can be used to identify a certain material.

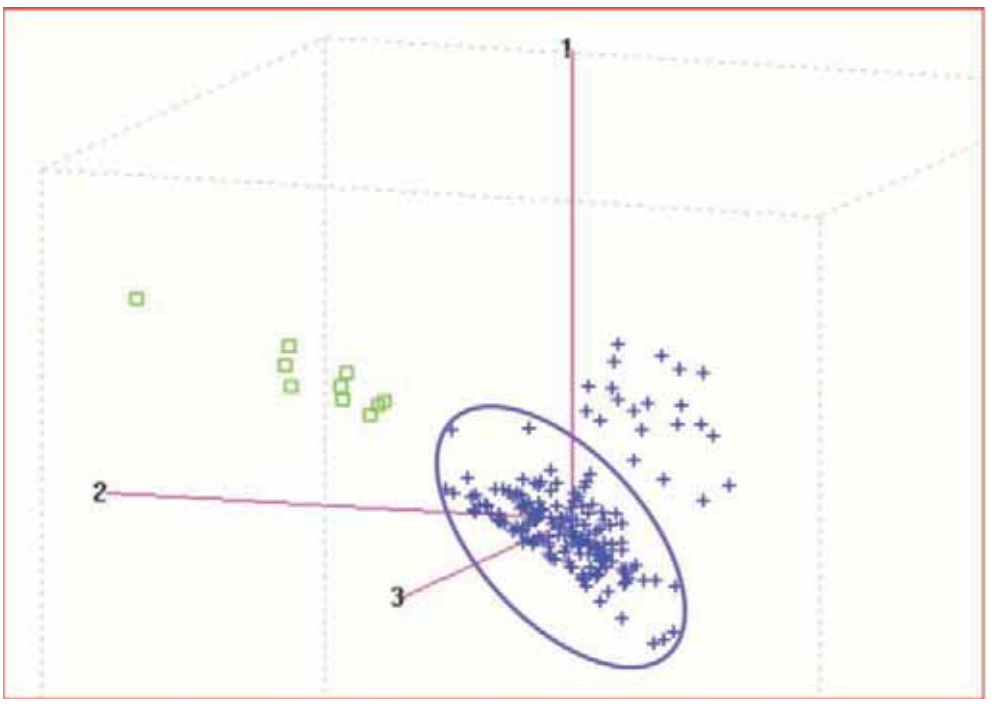

Figure 2: First three dimensions of the Principal Component Analysis of poultry meat. Samples marked in blue are samples from the control scheme, samples in green are adulterated samples. Blue samples outside the blue circle are poultry cuts (thighs, legs, etc.)

Near-infrared spectroscopy and food fraud detection

Near-infrared spectroscopy (NIRS) is one of the techniques that can be used for food authentication, either as a rapid alternative technique replacing a more laborious reference method or as a fingerprint approach. Its potential was discovered in the 1960s and the technique involves the measurement of a material's tendency to absorb light in a certain area of electromagnetic radiation. The resulting spectrum is a fingerprint of the material. The near-infrared portion of the electromagnetic spectrum extends from 800 to 2,500nm, between the conventional mid-infrared region at longer wavelengths and the visible range at shorter wavelengths. The NIR spectrum is characterised by overtones and combination of the fundamental molecular vibrations of molecules containing C-H (e.g. in carbohydrates), N-H (e.g. in proteins), or O-H (e.g. in water) groups. The absorbance level at these specific wavelengths is generally proportional to the quantity of that constituent in the material[3,4].

Traditional strategies for food authentication rely on the determination of a marker compound or compounds and the comparison of the obtained values with those established for genuine material

In view of food authentication, NIRS as a rapid alternative to targeted analysis with reference methods have been described to determine the major constituents of, for example, milk, milk powder, casein, butter and cheese with an accuracy similar to the accuracy obtained with the wet chemistry[5,6]; fat, water, protein, cholesterol, collagen and mineral contents of meat[7]; measurement of the iodine value of edible oils; caffeine in coffee; and ethanol in alcoholic beverages[4].

Furthermore, NIRS have been applied in the fingerprint approach to detect compositional adulteration e.g. orange juice adulteration[8], raspberry puree adulterations with apple puree[9] and adulteration of pomegranate juice with grape juice[10]. Other studies on compositional adulteration include the identification of cattle, llama and horsemeat by NIRS[11] and quantification of pork, fat trimming and offal in minced meat[12]. With regard to product history, some example studies demonstrate the use of NIRS for discrimination between organic and conventional production practices in asparagus production[13], the reflection of cow feeding regimes in the spectral characteristics of cheese[14], and discrimination between fresh and defrosted fish[15].

NIRS and food fraud example: single marker and fingerprint approach for poultry meat

As an example of the potential of NIRS for food authentication, the results of an investigation that has been carried out in the authors' research group will be shown. The water content of poultry meat varies with e.g. the birds' breed, age, and production parameters. The water / protein ratio is an important parameter in legislation regulating the import of poultry meat into the EU, and a limit of 3.40 applies for a chicken breast's water to protein ratio. The mean water and protein contents of poultry meat imported into the Netherlands analysed by wet chemistry reference methods has shown an increasing trend in water / protein ratio over the last decade (Figure 1, page 54). This may be due to a variety of reasons. Although the EU regulations prescribe the classical wet chemistry methods, rapid alternatives are useful for screening purposes.

In the present study, NIR spectra of a large group of poultry meat samples were generated. The spectral information was used to determine water and protein contents in poultry meat with the method

calibrated against the wet chemistry reference methods. Furthermore, the spectral data was used as fingerprints in order to distinguish groups of samples. Regular poultry meat samples (176) as part of an EU control scheme (EC Regulation No. 543/2008) and 10 chicken breast samples with water and retaining agents added were subjected to analysis. After grounding and homogenising, the samples were analysed for their moisture and protein contents according to the classical wet chemistry methods (ISO 1442 and ISO 937 standards), respectively. For NIRS analysis, samples were packed into a circular large sample cup (140mm Ø by 14mm depth) and placed into the NIR spectrophotometer[16]. The samples were scanned in transmission in the low near infrared region (from 850-1050nm). The NIR spectra of 151 chicken breast samples were then compared to those in a database which included over 20,000 sample spectra of different sample types and associated reference values and included poultry meat. The moisture and protein contents were calculated using the mathematical algorithms of the Artificial Neural Network model. Model predictions and wet chemistry results were compared. The NIRS results were quite reproducible and correlated well with the wet chemistry results. The accuracy of the NIRS results was similar to the accuracy of the wet chemistry methods and therefore, a rapid alternative for the wet chemistry methodology.

In addition, the NIR spectra of the total test set (186 samples of poultry meat) were subjected to Principal Component Analysis (PCA). PCA of the data revealed that all prepared samples were well separated from the regular samples (**Figure 2**, page 54). A centre group composed of chicken breasts was separated from the group consisting of poultry cuts (thighs, drumsticks, legs, etc.) with bones. The chicken breast samples with added water and retaining agents were separated from the regular chicken breasts in the second dimension which is promising in view of food fraud detection.

Other food authenticity studies of the authors' with NIRS include discrimination between wild and farmed salmon[17], general anomaly detection in milk powders with regard to nitrogen-replacers such as melamine, ammonium chloride, caprolactam, diammonium, phosphate and polyvinylpyrrolidone[17], geographical origin of coffee beans, and saffron adulteration.

Food fraud prevention future perspectives

In the future, when we have better understanding of the technological, criminological and situational factors that increase the risk of food fraud, dedicated food fraud assessments can be included in existing management systems. Rapid analytical detection techniques, which can screen for general anomalies in-/on-/at-line will help to control food authenticity. NIRS and hyperspectral imaging techniques are important techniques in this respect. Abnormal samples may then be subjected to further advanced confirmatory analyses in the laboratory.

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Rob Frankhuizen studied Analytical Chemistry at the Chemical College in Leiden and Delft and Environmental Control at the Technical College in Amsterdam. From 1969 to 1976, he worked as a chemical engineer at the The Rijnland District Water Control Board in Leiden. In 1976, he started as a Research Assistant and since 1992, has worked as a Scientist at the State Institute for Quality Control of Agricultural Products (which is at present RIKILT Wageningen University and Research Centre in the Netherlands) with the implementation of instrumental techniques and related analytical chemistry. Since 1980, he has carried out research in the field of quality control, authentication and processing of a wide variety of food and feed products using NIRS in combination with chemometrics. He managed the Product Composition research programme from 2006 to 2009. From 1992 to 2010, he also acted as an independent NIRS Consultant and Assessor for the Dutch Accreditation Council (RvA). Rob has published more than 40 scientific papers, written around 80 RIKILT reports and participated in numerous national, EU and global projects / committees / networks.

Saskia van Ruth has headed up the Authenticity & Novel Foods business unit at RIKILT Wageningen University and Research Centre in the Netherlands since 2012. This post doubles with her position as Professor of Food Authenticity and Integrity at Wageningen University. She received her PhD in Food Chemistry from Wageningen University in 1995 and carried out research on lipid and volatiles chemistry as post-doctoral researcher for Unilever in 1996 to1998. From 1998 to 2005, she carried out instrumental flavour research at University College Cork, Ireland and lectured in sensory science and related analytical chemistry. Subsequently she joined RIKILT in 2005 and managed the research cluster Authenticity and Nutrients, and has managed the Product Composition research programme since 2009. Saskia's research interests concern complex authentication issues with regard to production system (organic, sustainable, halal), geographical origin, processing, ingredients, and typicality (artisanal products) with application of state-of-the-art analytical methodology in combination with chemometrics. She has published more than 180 scientific papers and participated in numerous national, EU and global projects / committees / networks.

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Understanding the dark side of food: the analysis of processed food by modern mass spectrometry

Do we actually know what we eat? How well do we really understand the chemical composition of our daily food? These are two questions of utmost importance for consumers, food manufacturers and the scientific community involved in food research. The answer to that question is anything but straightforward. Our knowledge of the chemical composition of food resembles the Roman god Janus, on one side a tremendous knowledge has been built up over the last decade about the chemical composition of the raw materials used for food production. The database FoodDB contains around 28,000 entries of fully characterised chemical compounds found in our daily diet and for many food raw material food scientists are able to account for nearly every molecule present in the raw material.

The other side of Janus is processed food. Most of the food consumed by humans undergoes some sort of processing, either by thermal treatment (baking, roasting, frying, cooking, steaming, etc.), by fermentation, pickling, pressure treatment, irradiation or others. During these processing steps, usually associated with browning or darkening of colour (here referred to as the dark side), the chemical composition of the initial raw material is dramatically altered. The hundreds of components present in the raw material undergo a myriad of chemical transformations producing thousands of novel products. As a rule of thumb, it can be estimated that up to 50 per cent of the compounds present in the food raw materials are decomposed by chemical reactions to produce new products[1]. Since humans consume the vast majority of their food after processing, the actual percentage depending on social and cultural circumstances, up to 80 per cent of food consumed is estimated to be processed prior to consumption. It follows that the majority of the food consumed by humans is processed food.

It is worth pointing out that processing food constitutes a unique activity of humans and distinguishes humans from all other animal species on our planet. Very little is known about the chemical composition of processed food, mainly due to the enormous chemical complexity of the materials obtained after food processing, containing sometimes several tens of thousands of chemical compounds. Such large compound numbers are usually associated with the presence of an unresolved hump in a chromatographic analysis. These humps

with its associated complexity have so far constituted an insurmountable hurdle for analytical chemistry. Trying to gain a better understanding of processed food therefore means to develop and apply analytical methods that are capable of coping with such an enormous complexity.

Our research group has over the last few years developed such methods based on modern mass spectrometry that allow, for the first time, an understanding of the composition of processed food along with an understanding of the chemistry underlying food processing. This article will provide a short overview about the methods used and highlight some key findings of our investigations.

Modern mass spectrometry

Mass spectrometry has made some enormous advances over the past two decades. The advent of Electrospray Ionisation (ESI) and Matrix Assisted Laser Desorption Ionisation (MALDI) has allowed the soft ionisation of almost any biomolecule, large or small, stable or labile and its transfer into the gas phase for further analysis. Isolation of ions in the gas phase followed by fragmentation of intact ions in the gas phase produce fragment ions (this process is referred to as tandem mass spectrometry), which offer the possibility of carrying out structure detailed elucidation. Additionally, the coupling of mass spectrometers to chromatographic equipment and other spectroscopic techniques has allowed to separate compounds physically and obtain multi-dimensional information on the analytes, all this at relatively high sensitivity for routine commercial MS instruments in the nM range or better.

The key advantage of mass spectrometry in the analysis of processed food, however, is its resolution, in particular if using high resolution mass analysers such as Time of Flight (TOF) or Fourier Cyclotrone Resonance mass detectors (FT-ICR-MS), able to resolve tens of thousands of ions in a single mass spectrometry experiment. The resolution achieved in such experiments is therefore several orders of magnitude higher if compared to chromatographic separation or any other spectroscopic method.

A high resolution mass spectrum of processed food samples shows typically several thousands of resolved signals (see Table 1 for representative examples) e.g. around 2,000, for caramel, around 10,000 for a black tea infusion or around 30,000 for a cocoa powder sample. From the observed signals in the mass spectrum, molecular formulae for the large majority of signals can be determined, directly taking advantage of the high mass accuracy employed, providing extensive mass lists of all analytes observable. It should be noted that in such an experiment, not all analytes present can be ionised with one given ionisation technique, however, the use of complementary ionisation methods for example in positive and negative ion mode and using ESI, MALDI and APCI provides a more complete picture of the content of a given sample. Additionally, it must be noted that mass spectrometry is isomer blind at this stage, resulting in the necessity to obtain average isomer numbers from subsequent LC-tandem-MS experiments. These average isomer numbers are then multiplied with the number of signals obtained in a high resolution MS experiment, providing an estimate of real compound numbers. Figure 1 shows a typical MS spectrum of a complex mixture, in this case Maillard reaction products.

Table 1: Number of peaks observed in high resolution mass spectra in the negative ion mode for selected processed foods

Processed Food	Number of resolved peaks in HR-MS spectra at S/N > 50
Black tea	10 000
Fermented and roasted cocoa	30 000
Roasted coffee	2 000
Black pepper	5 000
Browned banana	3 000
Heated sucrose	400
Heated starch	300
Heated glucose	300

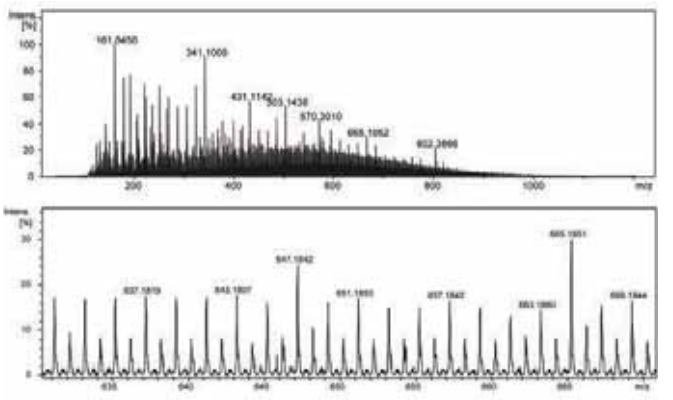

Figure 1: ESI-FTICR mass spectrum in negative ion mode of Maillard reaction extract; a) full spectrum; b) expanded region of spectrum

Interpretation of high resolution mass spectra

As mentioned above, a high resolution mass spectrum of processed food samples provides molecular formula information on thousands or even tens of thousands of analytes present within the sample in a single experiment. In order to interpret such extremely information rich samples, we have adapted and transferred data interpretation strategies pioneered in the field of crude oil analysis (petrolomics) along with the

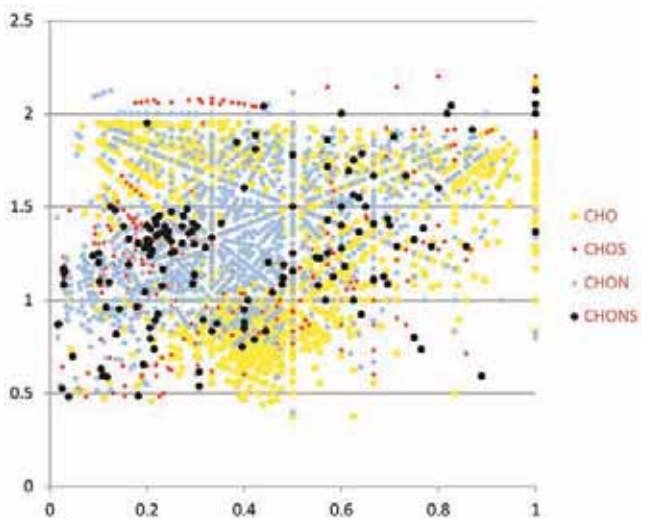

Figure 2: Van Krevelen diagram obtained from an ESI-FTICR mass spectrum in the negative ion mode of an aqueous cocoa powder extract. Ions of different elemental compositions are colour coded. Every of the 12,000 data points corresponds to a distinct analyte

development of novel data interpretation strategies. In petrolomics, two approaches have been shown to be of enormous use, the elemental ratio analysis (or van Krevelen analysis) and the mass defect analysis (or Kendrick analysis)[2].

In the van Krevelen analysis, elemental ratios e.g. H/C or O/C are calculated for each analyte from the molecular formulae information and plotted against one another. The beauty of this approach lies in the fact that certain classes of compounds (e.g. polyphenols, proteins, carbohydrates, lipids, terpenes phenolglycosides etc.) are characterised by a set of elemental ratio boundaries. Therefore, all members of a specified class of compounds appear in a restricted zone on the plot, hence a tentative classification of the tens of thousands of analytes in distinct classes of compounds becomes possible. **Figure 2** (page 59) shows a typical van Krevelen diagram of an FT-ICR-MS analysis of a cocoa powder extract with compounds of different elemental compositions colour coded.

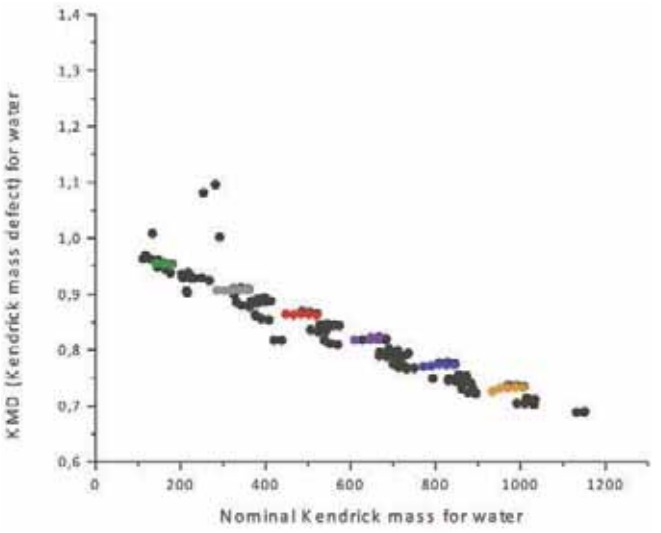

Figure 3: Kendrick diagram from an ESI-TOF-mass spectrum in the negative ion mode normalised for the H_2O mass increment for a caramel sample obtained from sucrose. Coloured points form lines parallel to the x-axis, which correspond to homologous series of compounds with stepwise loss of water (from right to left, up to eight water molecules are lost)

The Kendrick approach makes use of the observation that in a homologous series of compounds, which are defined as series compounds, to which starting from a given precursor, a defined group of atoms is repeatedly added. The repeated addition of a defined group of atoms with an associated mass increment leads within the series of compounds to a constant mass defect. If the plot is normalised to a given mass defect (e.g. in Figure x loss of water H_2O), all members of a homologous series lie on a straight line parallel to the x-axis on the Kendrick plot. Using this approach, homologous series of compounds can be readily identified from the data set. **Figure 3** shows a Kendrick diagram from a caramel sample, where homologous series formed by dehydration are shown in colour parallel to the x-axis. From our work, we could show that for nearly all classes of processed food, a relatively small selection of chemical reactions actually take place on heating (loss of water, addition of water, transesterification, transglycosylation etc.) or fermentation (oxidation, nucleophilic attack by water, carbohydrates, phenols etc.), always leading to a homologous series of compounds with repeated sequences of mechanistically identical reactions. Hence, the identification of homologous series immediately suggests the mechanistic pathways for chemical reactions occurring during food processing.

Further parameters can be derived from the high resolution MS data set and plotted against one another to provide graphical displays with patterns and trends embedded in the graph, whose visual inspection and later on rationalisation provides additional valuable insight into both the chemical composition and the mechanistic chemistry underlying food processing.

Chromatographic coupling to mass spectrometry

Liquid chromatography can be easily coupled to mass spectrometry and complex mixtures arising from food processing analysed this way. In contrast to the high resolution MS analysis by direct infusion described in the previous section, chromatography allows additional separation of isomeric structures and hence an estimate of the numbers of isomers present in a given sample. Additionally, the chromatographic separation reduces the effect of ion suppression and ion enhancement in a direct infusion measurement, which tends to distort the information obtained. Complex mixtures (also referred to as UCMs; unresolved complex mixtures) typically show next to well defined chromatographic peaks as an unresolved hump as exemplified in **Figure 4**.

If coupled to tandem mass spectrometry, additional structural information can be obtained from chromatographic runs. In the absence of authentic reference materials, which for processed food analysis constitutes a major problem, we have suggested that compounds of similar structure display identical fragmentation mechanisms. Hence from the homologous series analysis described tentative structural hypothesis for compounds produced in food processing must be developed, which in a second stage can be probed by LC-tandem-MS. A search in an extracted ion chromatogram reveals all compounds with a given molecular formula, and after inspection of their fragment spectra, these can be grouped into compound classes displaying identical fragmentation mechanisms. Establishing the correct regio- and stereochemistry for the compounds is of course not possible in the absence of authentic reference materials; however, to solve this problem we have used computational chemistry to aid compound assignment. Here, our approach is to use DFT calculations to obtain HOMO and LUMO orbital coefficients from starting materials in food processing, which immediately suggest likely regiochemical outcomes of the reactions occurring.

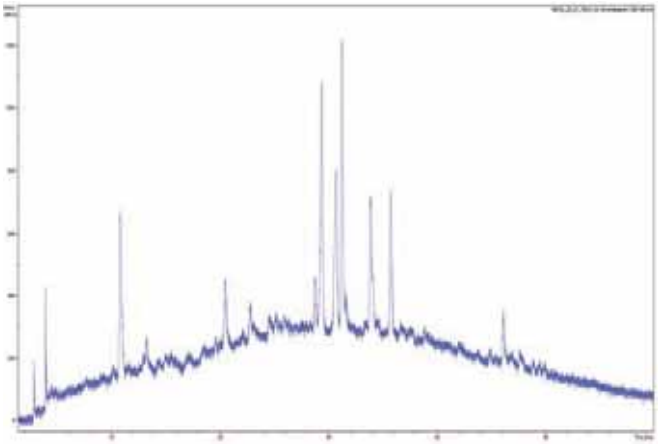

Figure 4: Typical HPLC chromatogram of a processed food (black tea infusion) displaying an unresolved hump

Structure elucidation by tandem mass spectrometry

MS has the advantage of being able to isolate ions of a chosen *m/z* value and subsequently fragment the chosen ions, revealing valuable structural information through the analysis of fragment ions. Recent years have shown that this method is much more powerful than previously assumed with even regio- and stereoisomeric ions yielding distinct fragment spectra that allow unambiguous structure elucidation. As a prime example, all four regioisomeric mono-esters of quinic acid and all six possible regioisomeric di-esters of quinic acid , all generally classified as chlorogenic acids, have been shown to display diagnostic and fragment spectra that allow predictive assignment of compounds[3]. Figure 5) shows the MS2 spectra of all six regioisomers of dicaffeoyl quinic acid as an illustrative example. Similarly, esters of diastereoisomers of quinic acids have been shown to display diagnostic fragment spectra allowing assignment of stereochemistry of the quinic acid moiety. In the case of quinic acid regioisomers and stereoisomers, all assignments of compounds could be supported with the aid of authentic reference materials obtained by chemical synthesis or extraction and isolation from natural sources[4].

In other work, we have shown that the same principle of structure elucidation can be applied to shikimic acid derivatives[5], lactones or proanthocyanidines[6] and in so far unpublished work, we have extended this principle to the unambiguous identification of carbohydrate esters and carbohydrates themselves.

In general, we take the view that tandem mass spectrometry is as information rich as NMR spectroscopy and should in principle be able to provide all information necessary for a complete structure elucidation of even complex structures. Two aspects, however, prevent a full exploitation of its tremendous potential; firstly a lack of standardisation of MS equipment and experimental parameters used in practice and secondly an insufficient understanding on how MS data are correlated to structure. Both will change within the next decade.

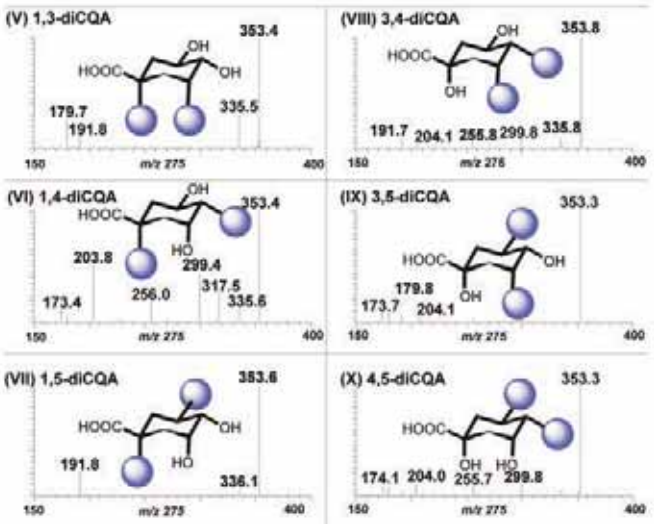

Figure 5: Tandem mass spectra of all six regioisomers of dicaffeoyl quinic acid (blue ball signifies caffeoyl ester substituent)

Quantification of compounds from Food Processing

Using our approach, no quantitative data on analytes present can be deduced from the data obtained, since in a complex sample, all analytes compete for ionisation and the ability to ionise preferentially determines the intensity of the signals observed rather than relative or absolute quantities. Additionally, the effects of ion suppression and enhancement

change signal intensities in samples of non-identical compositions. Hence for quantification authentic reference materials are always required, which after obtaining calibration curves can be used even in very complex samples for quantification purposes. Here, either tandem mass spectrometry in the single or multiple reaction monitoring mode (SRM or MRM) or the use of extracted ion chromatograms from high resolution LC-MS data can be employed, guaranteeing a high level of selectivity for the analyte of interest.

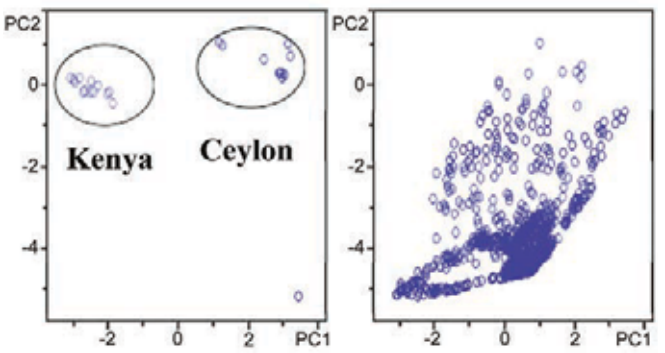

Figure 6: Score and loading plot of a PCA analysis of LC-ESI-TOF-MS data of Ceylonese and Kenyan black teas, allowing differentiation of the products in the score plot (left) and identification of analytes (every data point corresponds to one compound) in the loading plot (right) that are responsible for the differentiation

Data reduction using multi-variant statistical techniques

The section above described our strategies to carry out a full analysis of a chromatographic unresolved hump. Alternatively, one has the option to concentrate only on compounds that are important within a sample. The importance can relate to sensory properties, sample origin, food authenticity, processing parameters (improving visual appearance, shelf-life of a product etc.), beneficial health effects or possible IP considerations. If such parameters are related to differences in composition between samples, multi-variant statistical techniques can be employed to analyse a data set without loss of information. The most popular method to achieve data reduction without loss of information is principal component analysis (PCA). Here all analytes e.g. observed in an LC-MS run are characterised as a triplet of data containing retention time, *m/z* ratio and intensity. Out of all the data describing the analyte, a set of linear combination is computed (the principle component) and those searched that account for most variations between samples[7].

The results of a PCA analysis are displayed in two plots. Firstly the scores plot shows how sample groups can be distinguished in two selected PCs (each data point represent one sample or LC-MS run) and seconcdly in the loading plot every data point corresponds to one analyte that is in particular responsible for differentiations. Hence analytes that are important for differences between samples can be readily identified irrespective of the complexity of the original sample.

As an example, a PCA scores and loading plot of a series of thearubigins from Kenyan and Ceylonese black tea are shown in **Figure 6**. The scores plot allows clear differentialtion between samples, however the loading plot reveals the extraordinarily complexity of the samples with many hundreds compounds contributing to sample differentiation.

Selected examples of processed food analysed: black tea

Most of our analytical strategies for the investigation of processed food were developed for black tea chemistry. Black tea is produced from the young shoots of Camilla sinensis or Camilla assamica by a process referred to as fermentation. The green tea leaves are mechanically treated mixing their phenolic secondary metabolites, mainly six compounds of the class of the catechins, stored in the cell vacuoles with the enzyme polyphenoloxidase (PPO). PPO oxidises the B-ring of catechins to an ortho-quinone, which is subsequently attacked by a nucleophile, e.g. another catechin, to form dimeric structures such as theaflavins or theasinensins and a material referred to as thearubigin (TR) first described by E.A.H. Roberts in the 1950s. For structures, please see **Figure 7**. The chemical composition of the TRs remained mysterious and enigmatic for a long time and withstood for almost five decades any attempt to carry out a meaningful characterisation[8].

Using FT-ICR-MS, we could show that the TRs are composed from around 30,000 different compounds (10,000 signals in a mass spectrum multiplied by an average number of isomers) arising through the action of PPO acting on the only six catechins contained within the plant leaf. Using the analytical data interpretation strategies described, we formulated a hypothesis for TR formation, which has become known as the oxidative cascade hypothesis[9]. PPO produces electrophilic quinones, which are attacked by nucleophilic catechins to form oligomers (up to six catechin moieties). Additionally, water as the most abundant nucleophile in the green tea leaves furnishes polyhydroxylated derivatives of oligomeric catechins, which are in a redox equilibrium with their quinone counterparts. Hence 90 per cent of the TR constituents could be assigned with tentative structures and for more than 1,000 constituents, their structure was subsequently confirmed by tandem-MS. A sound and plausible mechanism for TR formation was introduced, which has so far withstood all further experimental tests[10].

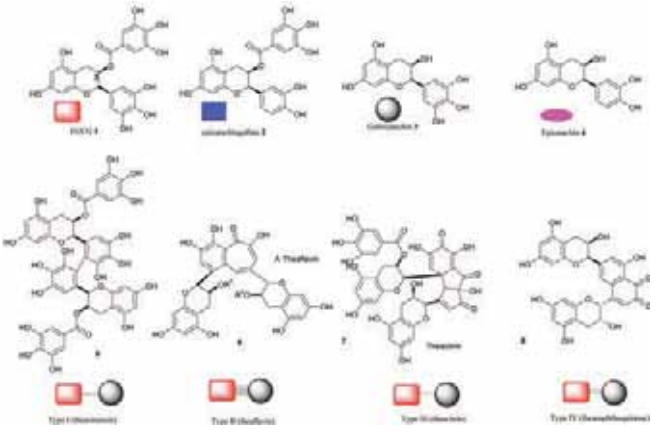

Figure 7: Structures of green tea catechins and dimeric oxidation products formed in the production of black tea by fermentation

Roasted coffee

Green coffee beans contain mainly a class of secondary metabolites termed the chlorogenic acids (CGAs) along with proteins, carbohydrates and lipids. Roasted coffee is produced by heating green coffee beans to temperatures ranging from 180 – 250°C for a period of 8 – 15 minutes producing different roasts. An FT-ICR-MS of roasted coffee extracts revealed the presence of around 2,000 signals, which could, by comparison to model roast systems, be classified into products arising from the reactions of all the individual classes of compounds present in the bean, with products arising from CGAs and carbohydrates

dominating the complex mixture. Using synthetic chemistry, we could show that CGAs undergo a series of simple organic transformation during roasting, which include acyl-migration, dehydration to form lactones or cyclohexene derivatives along with epimerisation. In a second brewing step, water undergoes conjugate addition to CGAs and subsequent beta-eliminations yielding cis-CGA derivatives[11]. Figure 8 illustrates the different mechanistic pathways observed for CGAs after thermal treatment in coffee roasting.

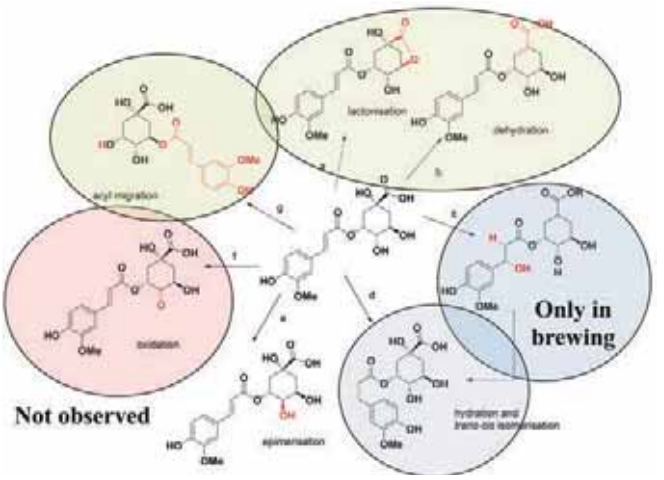

Figure 8: Structure of chlorogenic acid and typical reaction products of CGA identified in the roasting of coffee

Caramel

The products of thermally treated sugars are traditionally referred to as caramel. For caramel made from sucrose, we could show that a typical caramel sample is characterised by the presence of around 3,000 signals in an FT-ICR-mass spectrum. Further analysis revealed that structures encountered are formed by oligomerisation of sucrose and sequential stepwise dehydration. In contrast to CGAs, no epimerisation of sugar moieties could be observed[12]. Similar work was carried out for the analysis of thermally treated starch as frequently used in bread baking. Starch breaks down into small glucose oligomers followed by dehydration reactions resembling the chemistry of caramel formation[13].

Conclusion

In conclusion, our novel analytical strategies based on modern mass spectrometry allow, for the first time, a detailed insight into processed food, or otherwise referred to here as the dark side of food. The number of constituents present can be obtained along with molecular formulae lists for all constituents. Further data interpretation allows the formulation of structural and mechanistic hypothesis on the chemistry underlying food processing, thus a global picture of processed food composition emerges. We have so far successfully applied this approach to black tea chemistry, coffee roasting and caramel formation. Current work focuses on the investigation of cocoa production and the 100-year-old enigmatic Maillard reaction.

Our methods are not aimed at replacing the more classical approach based on isolation, purification and complete spectroscopic structure elucidation of food constituents. Rather, it constitutes a complementary approach to the classical way of food chemistry and should always be attempted in cases where thousands of analytes prevent due to poor chromatographic resolution their proper purification and characterisation.

We hope as well that regulatory authorities will take notice of our new approach and request and accept such data for the majority of processed foods, whether new or old, whose chemical composition has remained unknown and mysterious.

From a philosophical point of view, our work has highlighted a completely unexpected chemical complexity and chemical diversity of complex food, raising the general question on how humans are actually coping with a daily avalanche of tens of thousands of xenobiotics defining the chemistry and pleasure of processed food. We like to put forward the hypothesis that the chemical composition of processed food has largely contributed to the evolutionary success of the human species. To find out why and how will be the research challenge for the future.

About the Author

Nikolai Kuhnert studied chemistry at the University of Würzburg and received his PhD under the supervision of Professor W. A. Schenk in 1995. Following postdoctoral stays at the Universities of Cambridge and Oxford, he obtained his first faculty position at the University of Surrey. In 2006, he moved to Jacobs University Bremen, where he is now a Full Professor of Analytical and Organic Chemistry. His research interests cover the application of modern mass spectrometry in structure elucidation of natural products, the analysis of complex mixtures from processed food and the chemistry and biological activity of dietary polyphenolics.

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analytica 2014 – Gathering for industry and research

On 1 April 2014, it will be time again for the international community for laboratory technology, analysis and biotechnology meets at analytica in Munich. For four days, more than 1,000 manufacturers of laboratory equipment and analysis devices will present their latest products and highlights. The fair will revolve around the topics of food and plastics analysis as well as genetic analysis and bioanalysis.

Live Labs & Special Show: Occupational safety/ Health and safety in the workplace

Modern analysis techniques and innovative biological methods are now indispensible in most branches of industry – whether in the production of medications or quality control of drinking water. That is why analytica 2014 will revolve around food and plastics analysis as well as genetic analysis and bioanalysis. These topics will be reflected in the trade fair and its programme of related events as well as the Live Labs. Thirty-minute lectures and product presentations are on the agenda three times a day – at 11:00, 13:00 and 15:00. Among other things, they will deal with routine analysis applications as well as special analysis techniques.

Whether in research or industry, safety of laboratory employees is extremely important. Now analytica is integrating this topic into its Live Labs in Hall A3 with experimental lectures on how to avoid fires and explosions. It will also examine the best ways to protect employees' health in everyday laboratory operations and what measures are necessary to do so. The special show will also give exhibitors an opportunity to present products that are necessary for safety such as safety cabinets, gas detectors and protective clothing.

analytica conference

From pharmaceutical analysis, bioanalysis and clinical and medical diagnostics to environmental, water and material analysis: The programme of lectures at the analytica Conference covers the analysis sector in its entirety. The conference is held in conjunction with and on the first three days of the fair. For the first time ever, it is being held in the immediate vicinity of the fair, i.e. at the ICM – Internationales Congress Center München. Experts from Germany and abroad will use the conference to talk about the current state of the art and development prospects in analysis, biochemistry and clinical diagnostics. Among other things, topics will include Bio Metallomics, Bioimaging of Tissues and Proteomics in Biomedical Research Drug Testing and Metabolism in Samples Beyond Conve. Admission to the conference is included in the price of admission to the fair.

Forum Laboratory & Analytics

The latest applications and marketable products on the laboratory and analysis market will be presented in lectures and panel discussions in the Forum Laboratory & Analytics in Hall B2. Practice-oriented exhibitor lectures will give routine-analysis professionals tips that are relevant to business and actual applications. And on Friday, the forum will become a career springboard when analytica Job Day is held there.

Forum Biotech: Best practice for users

Life scientists use refined methods to complete the puzzle of life more and more, even though they often have just a few cells or a few microliters available for their measurements. As equipment manufacturers master these and other bioanalysis challenges, they report on their accomplishments in the form of Best Practice lectures and presentations in the Forum Biotech in Hall A3. The biotech region of Munich is considered a leading cluster for personalized medicine and targeted therapies. analytica places special importance on this future-oriented topic with an additional series of lectures as part of the forum. Lectures and panel discussions on recent and future developments and methods are planned.

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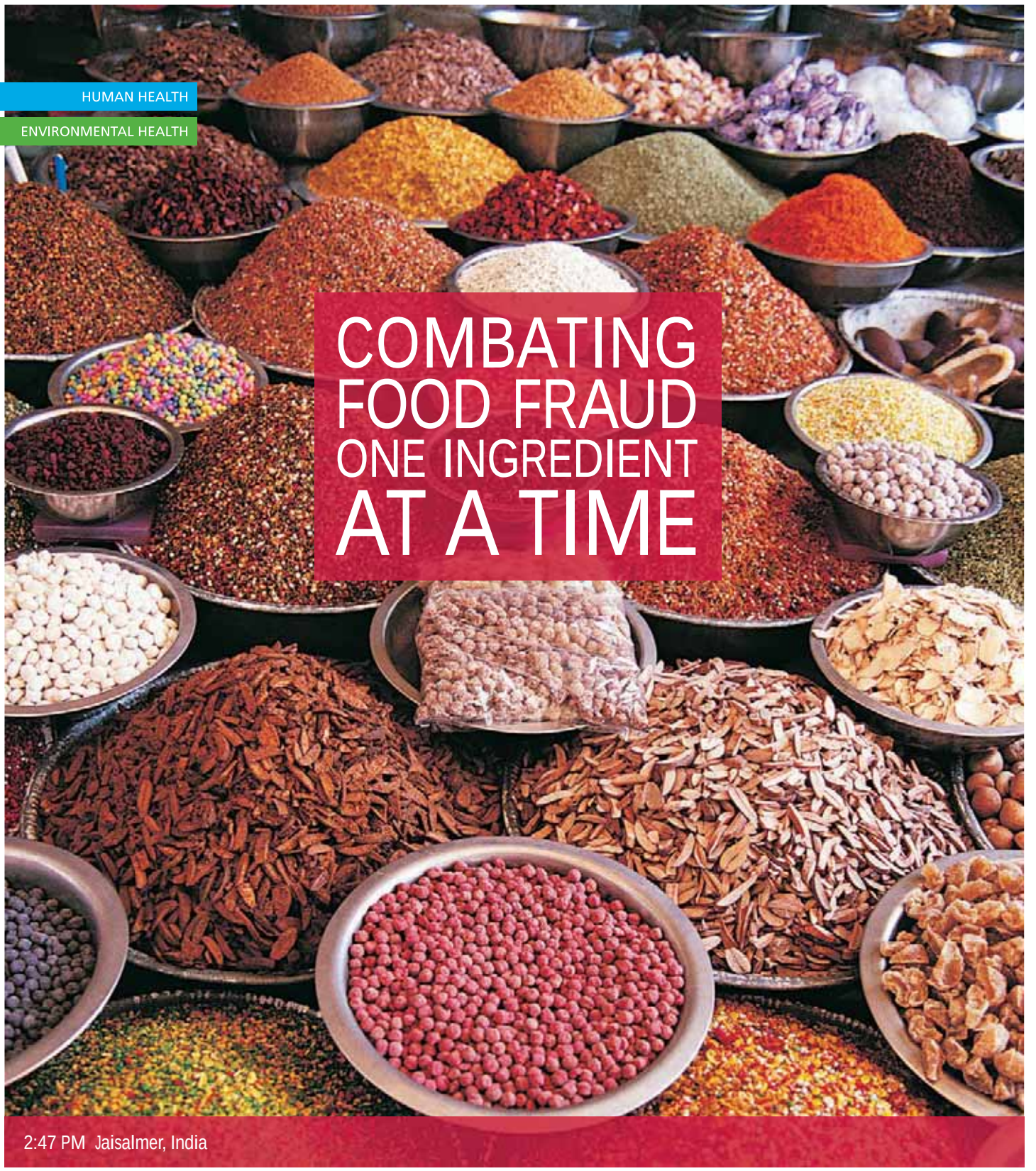

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